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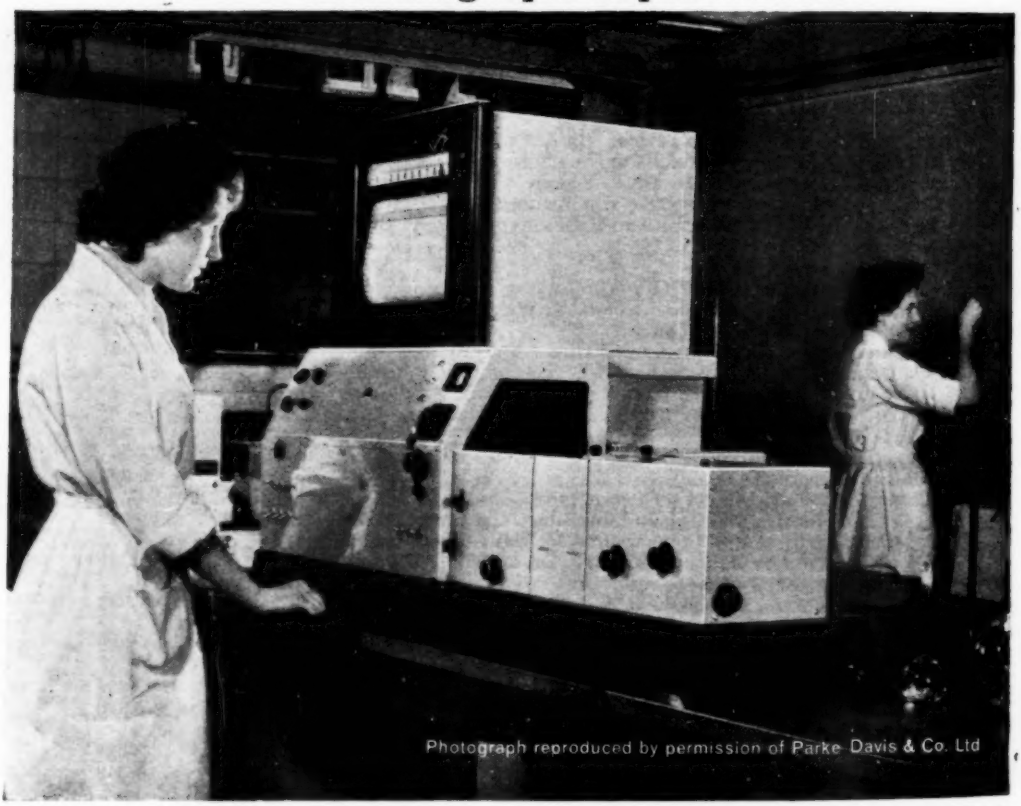

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SCIENTIFIC MAN-POWER IN BRITAIN

DURING the latter part of 1960, the Statistics Committee of the Advisory Council on Scientific Policy made a fresh survey of the long-term demand for scientific man-power in Britain, supplementing that previously made by the Council's Committee on Scientific Manpower in preparation for its survey of scientific and engineering man-power in 1956. This survey is intended to relate the subsequent expansion of educational institutions to the Council's plans for training scientific and technological man-power during the next decade and to the demands for employment which might be expected to arise by about 1970. This survey has now been published as an appendix to a memorandum by the Committee on Scientific Manpower*. Although the Statistics Committee did not regard the nature of the evidence at its disposal, particularly in relation to future demands for employment, as sufficiently precise for detailed conclusions to be drawn, the report should none the less form a useful guide to the Council in determining its general policy in training scientific man-power.

The Committee on Scientific Manpower points out that much might happen to invalidate the estimates made by the Statistics Committee, and the assumptions made will not necessarily be fulfilled. It is believed, however, that the present shortage of scientific man-power will continue for a long time in the educational profession, and in those disciplines which involve a high degree of mathematical skill. While the Statistics Committee has calculated the balance of supply and demand from its own assessment of the soundest of the alternatives before it, the report provides the material for a range of differing assumptions to be applied should further evidence become available.

In its terms of reference, the Statistics Committee was asked to assume that the university population would be raised to 170,000 by 1971, and that the projected increases in the output of qualified man-power for colleges of advanced technology and similar institutions would also be achieved. Its estimates of supply accordingly assume that suitable school-leavers will wish to enter such institutions in sufficient numbers to fill all scientific and technological places; that the present pattern of university output will continue (including lengths of courses and proportions of overseas students); that technological education will develop on lines at present foreseen (including present standards of professional qualification); and that the present pattern of migration, and loss by death, retirement, marriage, etc., will continue. The Committee on Scientific Manpower believes that in so far as these assumptions are closely linked with the physical limits of expansion, the report gives a substantially accurate picture of the numbers likely to qualify during the next decade, though changes in the pattern of output from the universities and in the lengths of courses are to be expected. It is assumed that the Committee's estimate in its earlier report of a total 'qualified' population of 72,200 scientists and 100,800 engineers in 1959 was reasonably comprehensive.

On these assumptions the qualified man-power available is expected to rise to 77,400 scientists and 100,800 technologists at the beginning of the 1960–64 period, 107,700 scientists and 147,300 technologists at the beginning of 1965–69, 149,300 scientists and 196,600 technologists at the beginning of 1970–74, and 206,000 scientists and 257,500 technologists in 1975. The numbers qualifying in each year are estimated as rising from 6,490 in science and 9,055 in technology in 1959–60, of whom 8,345 will be from universities and 7,200 from technical colleges, etc., to 8,660 in science and 11,620 in technology in 1964–65, 11,810 in science and 14,770 in technology in 1969–70, and 14,200 in science and 16,470 in technology in 1975–76, when 17,370 will come from the universities and 13,300 from technical colleges, etc. These figures are believed to provide a sound basis from which to consider possible demand, and stress is laid on the relatively low figure for wastage, due in part to the comparatively low average age of scientists and technologists in employment.

The assessment of demand has been accepted as essentially more hazardous than the calculation of supply, and in this review a more detailed assessment of each sector of employment has been used than in 1956 and of the main industrial groups separately. Moreover, the Statistics Committee has avoided restrictive assumptions and endeavoured to avoid significantly under-estimating the demand for employment or drawing too fine a balance between supply and demand. An exclusively vocational approach has also been avoided, and account taken of the tendency, which may well accelerate, for scientists and technologists progressively to invade fields of employment outside their disciplines, such as management, public services and elsewhere.

The estimates of demand depend on assumptions as to the programmes of research in the Civil Departments (particularly the research councils) and the expenditure on defence; the use of qualified man-power in each group in private industry; the ratio of scientists to technologists, and the proportion of the working population in 1970 in each industry; the requirements for teaching staff implicit in filling all planned and projected places in further education and in schools; and the form of the curve of demand between 1959 and 1970. As regards industrial employ-

* *The Long-Term Demand for Scientific Manpower.* Pp. ix + 26. (Cmnd. 1490.) (London: H.M. Stationery Office, 1961.) 1s. 9d. net.

ment, the most important assumption, made on the basis of inquiries, is that the speed of absorption of qualified man-power in technologically advanced industries tends to decline when a density is reached at which the technologies concerned are fully manned. Thereafter, the rate of absorption tends to be governed by the size of the industry and by replacement, until far-reaching but unknown technological changes create an entirely new industrial situation; but in a large industry there is reason for believing that technological 'break-throughs' as such do not normally involve taking on significant additional resources of man-power. The most advanced firms in certain leading industries in both the United Kingdom and the United States already appear to be exhibiting these tendencies, and the Committee assumes therefore that, for one economic reason or another, the density of employment of scientific manpower within these industries will rise to the level already characterizing the best firms in that group.

On these assumptions, and subject to the reservations indicated, the Statistics Committee concludes that the overall supply and demand for qualified man-power will not be much out of balance at the end of 1965. If anything, a slight shortage of technologists will be balanced by a slight surplus of scientists, and any possible divergence of supply and demand falls in the second five years of the 1960–70 decade. The survey suggests that the number of qualified persons per thousand in the working population can be expected to rise from 7 to 13 during the next ten years, and in many engineering industries as well as in the chemical industry, this doubling in a decade will be exceeded over the whole of the industry.

In presenting the report, the Committee on Scientific Manpower describes this doubling, with some justification, as in itself a potential technological revolution; and the report endorses the assumption that by 1970 there will be substantial improvements in pupil–teacher ratios and in the proportion of graduate teachers in the schools in Britain, notwithstanding big increases in the numbers of pupils in the higher forms and a doubling of the number of postgraduate students in the universities. Overall, the Committee's assumptions imply almost the same rate of growth of scientific employment during the next decade as has occurred in the years 1956–59; and the methods by which the estimates have been built up should command much readier acceptance of the conclusions, particularly in the present circumstances, than would have followed a simple projection of an existing trend. Indeed, it might be argued that this continued growth in scientific employment is an essential factor if Britain is to hold her place in the world's markets, independently of whether or not she enters the European Common Market, and not to decline, with all that that implies for her economic future and the standard of living of her citizens.

The conclusion that by 1965 the supply and demand of scientific man-power should be in balance and that, thereafter, a surplus may exist contrasts almost startlingly with the crippling shortages of scientific man-power that have been experienced since the Second World War and with the findings of previous reports on this subject. As the Manpower Committee points out, however, something can now be seen of the fruits of the efforts which have led to such a striking increase in our annual output of qualified scientific man-power since the War: from 10,000 in 1955 to 16,500 in 1961 and a prospective 20,000 by 1965 and 30,000 in 1973. It is true that the balance is a statistical one, and that supply and demand in individual disciplines, of which mathematics may be instanced as the most important example, cannot be so easily equated. Nevertheless, the Statistics Committee found no adequate basis for making separate estimates of the supply or demand for particular kinds of scientist or technologist, and the Manpower Committee recognizes that there may well be important differences in the factors which govern the prospects of employment between the specialized technologist and the engineer with a general training, and between technologists as a group and scientists, and that these problems remain to be examined.

In the view of the Committee on Scientific Manpower, the possibility of a surplus of scientists over immediate demands for employment should be welcomed. It should make possible a rational—as opposed to an emergency—use of the scientific disciplines; and it should mean that at long last Britain would have a supply of qualified man-power with a scientific training for management, administration and the professions generally in addition to those who up to the present have been drawn inevitably into vocational employment. The Committee does not doubt that scientific education will adapt itself to this new prospect, and that, just as only a proportion of those trained in the classics and history have expected to find employment in their own fields of study, an increasing proportion of those trained in specialized scientific disciplines will obtain employment outside them.

The Committee considers that both science and the nation will benefit from this adjustment and it believes that the universities are already thinking along these lines. If the Committee's confidence is to prove well founded here, it is certainly essential that the universities should not only think constructively but also be ready to act vigorously before the expected surplus arises. Modifications in the training of chemists and physicists such as have already been suggested from within the universities or by professional institutions could go far to correct the narrowness and rigidity of training which limit the employability of a scientist or technologist trained in a particular discipline; and too much care cannot be taken to ensure that neither the scientist nor the technologist is too narrowly trained. Colleges of technology, as well as the universities, may well have to resist very firmly pressure to give training in techniques which should properly be acquired in

industrial or professional practice, and in this context the growing stress being laid on a broad general education is to be welcomed.

The report, therefore, should administer a wholesome check to the tendency to excessive or premature specialization; but if waste of highly skilled man-power is to be avoided, there are other matters that require attention. The Manpower Committee is fully aware of the danger of complacency, and that the balancing of supply and demand is a relative, not an absolute, situation. The size of the reserves of ability on which Britain can draw, the problem of quality and the appropriate claims of disciplines in arts as well as science have to be considered; moreover, the calculations in the report do not cover medical, dental, agricultural or veterinary qualifications, the specialized technologies or the social sciences. All these must be considered in any national review, and will involve questions of transfer which may not be easy to forecast. Fully as important, however, are major questions concerning the relations, both in industry and in research, of qualified man-power and the technicians and other skilled man-power on which efficient use of professional man-power depends.

A report from the University Grants Committee, issued almost simultaneously with the report of the Committee on Scientific Manpower, directs attention to the low ratios of technicians to research workers in the universities, which is regarded as below that necessary to ensure that the time of research workers is used most economically. The Committee on Scientific Manpower hopes to examine this last issue more closely in the near future, but it is obvious that these questions of the conditions of service will not become any the less important if the nation experiences a surplus in place of a shortage of scientific man-power. Further, while the adaptability of the scientist and technologist becomes more important, whether in fact he or she is used in a wider range of occupations depends equally on the willingness of the employer to experiment. The scarcity of scientists and technologists in administrative appointments, for example, is not solely due to their reluctance to take up such posts. It is just as much, and possibly more, due to the unwillingness of departments or firms to give such appointments to the scientist or technologist; to the persistent belief that a person trained in a scientific discipline is inherently unfit for such responsibilities, though on this point it should be noted that the estimated demand of 12,400 scientists in the central Government by 1970 depends on the assumption that they will be increasingly employed outside the scientific field. No improvement in the general training of the scientist or technologist, or in the provision of opportunities for training for management, will avail while this prejudice persists.

The clear warning this report gives of a probable surplus of scientists and technologists within the next decade should stimulate fresh endeavours to deal with this and numerous other factors which hinder the wise, widespread and efficient use of science and technology in the ordering of British national affairs.

VISION, WITH AND WITHOUT TEARS

The Eye and Its Function

By Dr. R. A. Weale. Pp. vii + 209. (London: The Hatton Press, Ltd., 1960.) 55*s*. net.

Anatomy and Histology of the Eye and Orbit in Domestic Animals

By Prof. Jack H. Prince, Prof. Charles D. Diesem, Prof. Irma Eglitis and Gordon L. Ruskell. Pp. xi + 307. (Springfield, Ill.; Charles C. Thomas; Oxford: Blackwell Scientific Publications, 1960.) 126*s*.

The Structure of the Eye

Proceedings of the Symposium held April 11–13 during the Seventh International Congress of Anatomists, New York. Edited by George K. Smelser. Pp. xv + 570. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 107*s*. 6*d*.

THREE additions have recently been made to the already long list of books on the eye, and these all to a remarkable degree accentuate the structural aspects, but from very different points of view.

The first, *The Eye and its Function*, is addressed to "those who use the eye and wish to read about its function". Since all who are not blind use the eye for various purposes, the book is apparently intended for all those who wish to read about the eye. This category may obviously include all classes, from the film star interested in the practical use of the ocular muscles and the lacrimal glands, to the medical student, the research physiologist, the experimental psychologist, and even the greatest expert in ophthalmology. Naturally enough, the book reflects this lack of decision as to who are its potential readers and is a curious mixture of the commonplace, the significant and the abstruse. For the completely uninitiated there is probably too much technical information and knowledge required for the text to be easily comprehensible. For the expert in any one branch of the subject, the treatment is often inadequate and sacrifices of accuracy have sometimes been made in the interests of brevity or of an apparent, though possibly deceptive, simplicity. There is, however, a wealth of general information about the eye and its function to be gleaned from perusal of the contents. Both general biologists and medical students should find the book helpful and stimulating. In places, the English could be improved (for example, the legend for Fig. 9.3) and references to original sources are sometimes inadequate.

The second book, *Anatomy and Histology of the Eye and Orbit in Domestic Animals*, is clearly addressed to the veterinary profession, though the first five chapters, which give a good general picture of the eye and its associated structures, are based largely on human studies. The emphasis of the book is essentially on structure rather than on function, and special attention is paid to information on those features which are peculiar to the particular species investigated. The gross anatomy is, in general, better treated than the histology and cytology. Retinal structure, for example, is somewhat superficially treated.

The third, *The Structure of the Eye*, is a book for the specialists in cytology and vision. It incorporates the proceedings of a symposium held in New York in April 1960, and, unlike many books published

after similar symposia, is very informative and has both depth and breadth. Structure here includes mostly the molecular, cytological and histological, gross anatomy being scarcely mentioned. The book is an amazing compendium of the main new advances made in a very fertile field of research during the past decade or so. It includes excellent chapters on the microstructure, properties and functions of the photoreceptors, on the pigment epithelium, on the development, formation and properties of the lens, the vitreous body, the ciliary bodies and on the cornea. It is a book which is full of recently acquired information on all these topics and is thus of the greatest importance to all those who study the physiology of the eye and its visual functions, whatever their particular interest may be. Moreover, for cytologists in general, the problems of cellular structure and function which are discussed in this book must have an appeal far beyond the visual orbit. Indeed the structural basis for much general physiology is analysed within the pages of this book. The symposium was clearly very well organized and the fruit of it is well blended into a very digestible compôte. The book is very well produced and illustrated; it forms a valuable contribution to the literature on vision.

E. N. Willmer

LIGHT AND LIFE

A Symposium on Light and Life

Edited by William D. McElroy and Bentley Glass. (Sponsored by the McCollum-Pratt Institute of the Johns Hopkins University with support from the National Science Foundation.) Pp. xii + 924. (Baltimore, Md. : The Johns Hopkins Press ; London : Oxford University Press, 1961.) 120*s*. net.

THIS volume is the most recent addition to a series of symposia which have received acclaim for the authoritative character of the contributions and the breadth of the treatment which successive topics have received. The debt which biochemists and biologists owe them is evinced by the frequency with which they are quoted in the research literature, for they have provided both an accurate and comprehensive historical record of major fields of active research and a stimulus to further work and thought. A reviewer is compelled to appraise this recent addition to the series by the standards which its predecessors have set.

Against such a record, this volume appears somewhat disappointing. Difficulty is encountered at the outset in that the subject appears to have no natural unity. Three groups of biological phenomena are discussed : the emission of light in bioluminescence, the utilization of light in photosynthesis, and the photosensitization of certain processes, including phototropism, photoperiodism and vision. Some correlation between these diverse activities might be expected in the physicochemical character of their primary reactions and the molecular changes associated with light absorption or emission, but at this stage the gap between our knowledge of the biological and chemical features is so wide that the two groups become almost autonomous, and individual papers even within sections can appear little related to any other. Our ignorance is too great ; our knowledge cannot provide the cement to hold them together, and imagination has yet to come to our aid.

The arrangement of the contributions is rational enough. The response of molecules to photo-excitation is discussed first, with treatment of the excited states of simple molecules, the nature and importance of the biologically interesting $n \rightarrow \pi^*$ transition, and the triplet state. The general conclusions are then applied to the specific properties of biologically important molecules, including proteins, and pyridine and flavin nucleotide enzymes. This section, however, deals with the application of spectral data to structural and kinetic problems, rather than the biochemical consequences of the excitation of these molecules.

Chemical and biological aspects are wedded most effectively in the section on bioluminescence. Here, the chemistry of luminescence in the porphyrin-catalysed decomposition of peroxides and of the luminol reaction is described and discussed before proceeding to the various bioluminescence phenomena, ranging from the firefly and *Cypridina* to fungi, the dinoflagellates and the sea anemone. The comprehensive paper on firefly luminescence by McElroy and Seliger is particularly valuable.

Extended discussions on photosynthesis have now become so frequent that contributors must experience difficulty in sensing positive achievement year by year ; the same arguments can be rehearsed too often before the same public. Certainly, much that appears here has already been published. Three long articles—on the spectral character of chloroplast pigments, the immediate consequences of chlorophyll excitation, and the biochemistry of generation of adenosine triphosphate in chloroplasts and bacterial chromatophores—dominate this section, which gains interest from the papers discussing electron spin resonance studies, and the likelihood and consequences of more than one primary photochemical reaction sensitized by different pigments. Some of the other contributions are rather slender and appear to deal with reactions too remote from the primary action of light to justify inclusion in a symposium under this title.

The final section deals with photosensitized phenomena in biology, in which both the initial activation and the amplification necessary to achieve the response, have to be considered. Phototropic curvatures and photoperiodic responses have still not progressed far beyond the descriptive stage, and critical experiments which contribute to an appreciation of their mechanisms have yet to be carried out. The contributions by Rushton and by Wald on the molecular character of the excitation process in vision and its consequences set a pattern for the treatment ; two interesting but inconclusive papers on colour vision in insects and goldfish conclude the symposium.

The discussions after lectures, with their mixture of information, wit, boorishness and irrelevancy, have been included, though it is regrettable that the comments on some papers have been recorded before their delivery (or publication). The whole symposium is summarized *in extenso* by Bentley Glass, usually accurately and informatively, so that those not minded to read each paper can obtain the gist with less effort.

Even though the organization and content of the papers in this volume may be subject to some criticism, any who read this volume thoroughly cannot fail to be impressed by the range and variety of the information and to be stimulated to integrate it each after his own taste.

V. S. Butt

SELECTED STUDIES IN CHEMICAL KINETICS

Selected Studies in Chemical Kinetics
By Prof. Farrington Daniels. (Thirty-fifth Annual Priestley Lectures.) Pp. vi+123. (University Park, Penn.: Phi Lamda Upsilon and the Department of Chemistry, Pennsylvania State University, 1961.) 2.75 dollars.

WHEN considering a new plant for the chemical process industries the three important questions that must be answered are how big, how much and when. In approaching the problem of how big, knowledge of the rates of reaction or kinetics and the conditions of equilibrium are two vital tools of design. Prof. Daniels, in the 35th Priestley Lectures, sets out to give us an indication of the progress made and the general techniques available in handling the problem of kinetics.

The general arrangement of these lectures is to give initially an outline of the principles of kinetics and the relation of this subject to other sciences. This is followed up with a number of illustrations of work in this field primarily conducted by Prof. Daniels and his associates. It is by these examples that Prof. Daniels succeeds in indicating the methods of attack that are possible and at the same time makes it clear how difficult this subject really is. Kinetics, as understood here, deals primarily with the time and mechanism of a chemical reaction. It includes such subjects as catalysis, photochemistry and spectroscopy. It is important to realize that when two or more compounds react they give rise to several different products but the one with the largest yield is that produced by the fastest reaction. It is becoming both a science and an art to adjust the temperature, concentration, catalyst and solvent so as to favour the desired reaction to the maximum extent. For industrial purposes all reactions must be made to proceed fast enough to be economically feasible, and this is often a vital problem. At the same time, although kinetics is essential in industrial design it also offers an intellectual challenge, as, in the words of Prof. Daniels, "We have to make the molecules do our bidding".

The first main illustrations cover the work on the decomposition of nitrogen pentoxide. Here it is interesting to read how the mechanism in this reaction has proved a considerable puzzle and the sorting out of this complex reaction has provided many opportunities for developing new techniques in experimental work. The second illustration refers to the oxidation of nitric oxide, again a reaction of enormous chemical importance where the slowness of the reaction has presented problems in engineering design. This is a particularly interesting reaction as it is not only termolecular, which is relatively rare, but also has a negative temperature coefficient.

Other reactions discussed in some detail are the decomposition of nitric oxide and the Wisconsin process for fixation of atmospheric nitrogen. The account of the work is interesting both in terms of physical chemistry and chemical engineering, where the application of sudden chilling of the gases by pebble beds is relatively new and remarkably effective.

In the final lecture, Prof. Daniels refers to the kinetics of reactions in solution and in a solvent. He offers some useful information on some of the latest techniques used in studying these reactions, and his chapters on photo-kinetics and photo-chemistry are very interesting reading.

This set of lectures is welcome as it provides an extremely valuable account of the importance of kinetics in tackling industrial problems where design of reactors has proved more difficult than the design of much of the equipment used for physical separation. The lectures are of value to chemists and to chemical engineers.

J. M. COULSON

MATHEMATICAL THEORY OF NUTRITIONAL RELATIONSHIPS OF FISHES

Experimental Ecology of the Feeding of Fishes
By Prof. V. S. Ivlev. Translated from the Russian by Douglas Scott. Pp. viii+302. (New Haven, Conn., and London: Yale University Press, 1961.) 7.50 dollars; 60*s*.

THIS book, by a leading Soviet experimental ecologist, constitutes an important contribution to the nutritional ecology of fishes. Many factors govern the rate of feeding of a fish, and also its choice of particular foods in selective feeding. They include the total density of food, its aggregation, the partial densities of its separate components, the protection afforded to the prey by refuges or by mobility, the amount of contact with other fishes, the formation of habits of grazing on particular foods. These factors influence the fish simultaneously, so that the analysis of any one of them under natural conditions is practically impossible. In a long series of carefully controlled laboratory experiments, Prof. Ivlev has been able to isolate the factors one by one, and to investigate the influence exerted by each acting alone. Then, by means of a graded series of recombinations of the factors, he builds up a system of formal equations which represent not only the quantitative changes in the diet of the fish but the qualitative ones as well, very closely.

Ivlev's approach is essentially biophysical. He has modified the classical approach of Lotka and Volterra to the question of the effects of the concentration of the food on the rate of feeding, by introducing a factor which inhibits unrestricted increase in the rate of feeding at higher food concentrations. This leads him to apply the monomolecular or inverse exponential equation. This equation satisfies the experimental data very closely.

A large part of the book is concerned with the expression of selective feeding. Here Ivlev adopts a new type of coefficient of selectivity, which works well in a variety of situations. It can also be applied to data suitably collected under natural conditions.

Ivlev is the first to have published such an experimental analysis of these problems; I believe that the publication of the work in English will stimulate many new investigations into outstanding problems in this field. It may be noted in passing that all the experimental animals used were freshwater fishes; as regards marine animals, the field is wide open.

Considering the importance of the book, the standard of translation is regrettably poor. The Yale edition contains the most shocking mistranslations; there are misleading blunders on every page. while the text has scarcely been rendered into good English. The author's style is laconic. and it is difficult sometimes to find apt ways of rendering it; this is no excuse for writing the first thing that comes

to mind. Editing plays no small part in producing a work of this nature; manifestly, this translation has not been edited at any stage. For the garbled result, the blame rests equally on Mr. Scott and the Yale University Press. It certainly does no credit to either.

In view of these latter considerations it might be thought that the stipulated price is inordinately high.

L. Birkett

PROGRESS IN CANCER RESEARCH

Cancer Progress, 1960

Edited by Ronald W. Raven. Pp. xv+242+16. (London: Butterworth and Co. (Publishers), Ltd., 1960.) 60*s*.

ONE of the problems of the organization of research, particularly in the cancer field, is to steer between the Scylla of lack of co-ordination and the Charybdis of too much. When the ship sails too near the latter, senior workers find themselves spending almost all their time attending committees and meetings and writing review articles, while junior workers, who are in any event forced to specialize too early, rarely find the time to read review articles outside their own subject. Regrettably, it is with increasing frequency that we see the same data presented in more than one paper, and worse still, the same fields of investigation reviewed more than once by the same individual.

Against this background the editor, Mr. R. W. Raven, and his advisers, are most certainly to be congratulated both on their choice of well-defined subjects and on their selection of authors. The brevity of most of the contributions, and the fact that many of them are presented as sequels to chapters in the parent book, *Cancer*, gives this volume an entirely different form and function from *Advances in Cancer Research* or the *Year Book of Cancer*.

As one would expect, the standard of contributions varies considerably. Articles on tumour viruses (R. J. C. Harris), bone marrow replacement (H. E. M. Kay and P. C. Koller), circulating cancer cells (J. W. Stewart), carcinomatous neuropathies (D. S. Russell), carcinoid tumours (P. J. D. Snow), industrial causes of cancer of lung, nose and paranasal sinuses (P. L. Bidstrup), the effects of radiation on the normal lung (T. J. Deeley) and the effect of oxygen in radiotherapy (I. Churchill-Davidson) are among the many interesting and useful contributions.

Russian authors (N. I. Lazarev and L. F. Larionov) contributed three of the five chapters in the section on chemotherapy. In one of these it is cogently, though somewhat naïvely, argued that both the carcinogenic and the anti-tumour effects of hormones rest on their physiological activities. The last chapter in this section (D. A. G. Galton) corrects any impression given by the previous four, that chemotherapy almost holds the key to the cure of cancer.

In an interesting article on cancer in the South African Bantu, A. G. Oettlé describes the enormous difficulties in obtaining a clear picture of the incidence of cancer in a primitive society. This is a timely warning to those who criticize civilized practice on the grounds that cancer is infrequent among communities living a more 'natural' life. With regard to treating a member of such a community, he writes, "His fears and prejudices, his misunderstandings and his phobias have all to be considered in the therapy of the whole man". Such is equally true treatment of less-primitive men.

Among the least pleasing features of the book are the fairly small size of the type and the narrow width of the margins: it is hoped that this form of economy will be dispensed with in future volumes.

F. J. C. Roe

MEDICINE AND INSECT PARASITES

Medical Entomology

By Dr. William B. Herms and Dr. Maurice T. James. Fifth Edition. Pp. xi+616. (New York and London: The Macmillan Co., New York, 1961.) 87*s*. 6*d*.

THE fourth edition, by the senior author only, was published in 1950. This fifth edition is the work of Prof. M. T. James, who has clearly, with much care and endeavour and with considerable success, produced a book several pages shorter than its predecessor yet combining a good deal of its original format and material with new matter. This includes both new text, which brings the book more up to date in many aspects of the subject, and new illustrations, which enhance the pictorial presentation of its topics. As noted in the preface, veterinary entomology has been pruned considerably and the sub-title of the fourth edition, implying this aspect, has been dropped. Nevertheless, sufficient remains to serve the medical man who might well, in rural communities, have to think broadly, especially of mites and ticks, œstrids and biting flies.

In general, trends in control are well revised regarding contact insecticides and repellents. Malaria is discussed, with leading references, in terms of control and world eradication—for which the book observes a cautious optimism. The mathematical approach to the epidemiology of malaria, as analysed by Macdonald, is indicated usefully and briefly. Due elaboration is given to more recent discoveries on arthropod-borne virus diseases transmitted by mosquitoes and by ticks. Revision of the housefly and its allies is commendable and, although the section on tsetse is barely altered, the reader is led to the newer important general works on this subject. Nomenclature has usually been brought into line with recent authoritative works. There are, of course, points of criticism: Garnham is spelt Garman: there is no indication of the use of dieldrin for triatomid bugs; the section on loiasis makes no mention of the important ecological studies of this disease in the past decade, nor is the association of *Simulium* larvæ with crabs noted; there could be more about *Mansonia* mosquitoes.

Of the several keys, some new, those referring only to American fauna (for example, mosquitoes, *Culicoides*) must have limited interest, but others (for example, on fleas, ticks and mites) will have wider utility. It is difficult to see how, in a general work, such selection can be avoided.

Many sections are simply reprinted, but much new information is included in the re-arrangement of chapters and within chapters, the new format providing much clearer printing of sectional headings. The reader is assisted to main discussions of particular topics by bold-face page references in the index.

This is a worthy successor to the four previous editions and should prove an informative and useful general work for the student, and teacher, of medical entomology.

D. S. Bertram

ADVANCES IN THE GENERATION AND USE OF VERY HIGH, CONTINUOUS MAGNETIC FIELDS

By Dr. HENRY H. KOLM

National Magnet Laboratory*, Massachusetts Institute of Technology, Cambridge, Massachusetts

A MAGNETIC field exerts four effects which make it useful as a research environment: it produces a torque on a magnetic dipole, it exerts a force on a moving charge, a space-varying field exerts a force on a magnetic dipole, and finally a magnetic field varying with time exerts a force on a stationary charge.

When a magnetic field is applied to the microscopic dipoles in various solids, such as paramagnetic salts for example, the dipoles do not simply become aligned with the field, for two reasons: ordering is opposed by thermal motion which tends to preserve random orientation; in the second place, these magnetic dipoles also possess angular momentum and therefore tend to behave like gyroscopes; instead of aligning themselves with the direction of field, they precess around it at their precession frequency. The alignment of dipoles against thermal agitation produces reversible heating, a mechanism which is utilized in achieving the lowest possible temperatures. The precession frequency of gyroscopic dipoles is observable as absorption in the microwave or infra-red frequency-range, and provides valuable information concerning electronic band structure, crystalline magnetic fields, etc.

The second effect, the force exerted on moving charges, forms the basis of all particle accelerators in which a magnetic field is used to confine the accelerated particles within circular orbits. This confining force is loss-less, since a magnetic force always acts perpendicularly to the direction of motion. Magnetic fields can therefore also be used to confine very hot plasma, or ionized gases, by preventing the energetic particles from ever giving up their energy to the material walls of a physical container. They are also a valuable tool in producing a measurable curvature in the trajectory of high-energy particles. Space-varying fields, or fields with a high gradient, are used to measure magnetic susceptibilities, and also to analyse beams in experiments with atomic beams.

Finally, the force produced on a stationary charge by a field varying with time simply represents the law of electromagnetic induction, which forms the basis of electric motors and generators, as well as radio propagation. The effect is also used experimentally for accelerating particles in circular accelerators and for heating plasmas in fusion reactors. Thus we see that a magnetic field is required for producing the lowest as well as the highest attainable temperatures. Both these applications require magnetic fields of reasonably large volume, while solid-state research requires primarily fields of high intensity but modest volume.

Laboratory magnetic fields are conventionally produced by iron electromagnets which consist of an iron frame, one or two legs of which are surrounded by coils. The field induced in the iron is approximately 1,000 times as intense as the field which would be produced by the same coils in the absence of iron, and it is made accessible for experimentation by introducing a gap or break in the iron frame. This method fails if a long or prolate field volume is required, because an excessively long gap in the iron core eliminates the effect of the iron; the method also fails if the required field intensity is higher than the value at which iron becomes saturated magnetically. In both these cases it is then necessary to generate the field without the use of iron, or with only incidental assistance from iron. The elimination of iron requires approximately 1,000 times as much current, which makes the generation of very high continuous fields a difficult and costly undertaking. Although thermodynamically it should require no expenditure of power to maintain a magnetic field, it is an ironical fact that this is the only process we perform with zero efficiency. It is necessary to remove all the power supplied to a magnetic coil in the form of heat. Recent progress in the development of superconductors capable of withstanding substantial currents and magnetic fields suggests that we may actually be on the verge of eliminating this thermodynamic incongruity.

It is much simpler to produce high fields for very short periods of time by using stored energy and relying on thermal inertia to keep the coils from melting. Work with pulsed fields was pioneered at the University of Cambridge in 1927 by Kapitza[1], who achieved 500 kilogauss for periods of milliseconds by using energy stored in batteries and in rotating generators. More recently, the technique has been advanced into the megagauss range by the use of capacitor energy-storage and improved coil design[2,3] and by harnessing explosive energy[4,5].

The earliest effort at generating very high continuous fields was undertaken at the Massachusetts Institute of Technology in 1936 by Bitter[6]. It was stimulated by the need for more resolution in optical measurements of the Zeeman effect during the pioneering days of spectroscopy, and by the need for achieving lower temperatures by adiabatic demagnetization. The magnets developed by Bitter are helices formed by stacking copper disks with a radial cut, interleaved with sheets of insulation. Neighbouring copper disks overlap at their radial cuts so as to establish pressure contact when the entire stack is compressed. The copper is cooled by direct contact with water, which flows through some six hundred small holes passing through the entire stack in the axial direction. Bitter succeeded in generating 100 kilogauss in a helix of this sort having a 1-in bore and an outside diameter of 16 in., by supplying it with 1·7 MW. (10,000 amp. at 170 V.) of power and 800 gallons per min. of cooling water. Similar installations and magnets have been built since in several laboratories

*Supported by the Air Force through the Air Force Office of Scientific Research.

throughout the world, but for more than twenty years no effort was made to advance beyond the 100-kilogauss level. This is due largely to the fact that interest in obtaining higher continuous fields was relatively mild and de-centralized among so many widely separated scientists that no concerted effort of the required magnitude was made.

The situation changed quite suddenly during recent years with the advent of several vital experiments which use magnetic fields as a tool for investigating the nature of charge carriers and their energy-band structure in solids. The most prominent among these is the observation of 'cyclotron resonance', a phenomenon based on the circumstance that all particles (or holes) having the same charge-to-mass ratio will orbit around the flux lines of an applied, continuous magnetic field at the same frequency, regardless of their individual energy: faster particles will simply describe larger orbits than slower ones. This coherent behaviour of statistically distributed but identical particles manifests itself in resonance absorption at a frequency varying with the applied magnetic field and situated in the short microwave region when the magnetic field is in the range 20–30 kilogauss. These experiments[7] could only be performed in crystals of great perfection (originally germanium and silicon) in which the mean free path of charge carriers is large compared with the cyclotron orbits, because otherwise scattering by collision occurs so frequently as to mask the resonance. In order to observe cyclotron resonance in materials of less perfection, it was clearly necessary to increase the frequency from the shortest usable microwave to at least the longest usable infra-red. Unfortunately, this made it necessary to increase the magnetic field from the 30 kilogauss range, readily available in iron electromagnets, to beyond the 100-kilogauss range. The first attempt to do so was made by the Lincoln Laboratory group in 1955 (refs. 8 and 9), using a pulsed magnet capable of up to 750-kilogauss pulses of 120 μsec. duration, in a hole 3/16 in. in diameter. It was energized by an underdamped (that is, oscillatory) capacitor discharge so as to minimize resistance losses and permit the use of coils made of alloys having greater strength but also higher resistance than copper. Problems involved in the rapid detection of changes in infra-red transmission or reflexion presented some difficulties and limited the accuracy of measurements, but valuable exploratory results were obtained for InSb, InAs and Bi. Quite incidentally, certain oscillatory effects were also observed which were eventually resolved and studied in continuous fields.

This early experience emphasized both the importance of working in higher fields and the inadequacy of pulsed fields, particularly in the farther infra-red region, where the decline in both source intensity and detector sensitivity requires long measurements. It appeared imperative for a number of other good reasons as well that a new attempt be made to advance the art of generating very high continuous fields beyond the point at which it had remained for such a long time.

It was in this spirit that, with several other members of the Lincoln Laboratory Solid State Division at the Massachusetts Institute of Technology, notably Benjamin Lax, Donald Stevenson and D. Bruce Montgomery, I joined forces with Francis Bitter for a renewed attack on the old problem. Three efforts were launched at about the same time: first, the problem of designing high-performance solenoids was re-examined in the light of all available information; secondly, a proposal was made and presented to a number of Government agencies and leading scientists suggesting that a new laboratory be built for the purpose of making generally available for research the highest continuous magnetic fields which can be generated at the present state of the art; thirdly, communication was established among scientists concerned with the generation and use of such fields. I propose to report briefly the progress made along these three lines during the first three years.

The problem of designing solenoid magnets is best characterized in terms of a relation discussed extensively by Bitter, namely:

$$H = G\,(W\lambda/r\rho)^{1/2}$$

where H is the field at the centre in gauss, W the power dissipated in watts, λ the 'space factor' or fraction of solenoid volume occupied by conductor (as compared with insulator and cooling channels), ρ is the resistivity at operating temperature, r the inner radius in cm., and G is a geometry factor (the 'Fabry factor') which characterizes the current distribution; this factor is 0·179 for a uniformly wound solenoid of optimum dimensions and about 0·209 for a helix such as the Bitter magnets, in which the current distribution is highest near the centre and falls off inversely with the radius. When generating fields up to about 200 kilogauss, it is possible to maximize G by selecting the best realizable current distribution, but beyond this point it is necessary to depart from the optimum distribution in order to prevent mechanical failure of the windings near the centre. The space factor and resistivity must be considered together, since they are both governed by cooling, the former by the amount of cooling area required and the latter by operating temperature. Operation at cryogenic temperatures might appear attractive at first sight, but unfortunately as the temperature is lowered the required cooling area increases much more rapidly than the resistivity decreases, so that magnets become progressively inefficient. Only if fields of very large volume are required or if operation is to be brief and infrequent do the economic factors favour cryogenic cooling. Water offers considerable advantages even under conservative transfer-rates, say, 200 W./cm.^{2}; if local boiling in sub-cooled water is permitted, it has been found possible to transfer as much as 5,000 W./cm.^{2}, a rate which exceeds the rate of heat transfer at the surface of the Sun. After verifying experimentally that transfer-rates of this order of magnitude are indeed feasible, and that they can be maintained with stability in very small channels (about a quarter millimetre thick), we undertook our first concrete task, that of building a magnet which would produce a higher field than the old Bitter magnets with the same amount of power. It was intended not only as a research tool for immediate use but also primarily as a means for exploring new solenoid structures and new levels of performance.

Since the helical structure used in the Bitter magnets does not lend itself to reduction of size without loss of heat transfer area, we selected a tape-wound spiral structure with funnelled ends for optimized current distribution. Successive windings are separated by ribs which are aligned along radii like spokes of a wheel so as to transmit the radial outward forces to a strong confining envelope. Cooling is achieved by axial flow through the channels between these

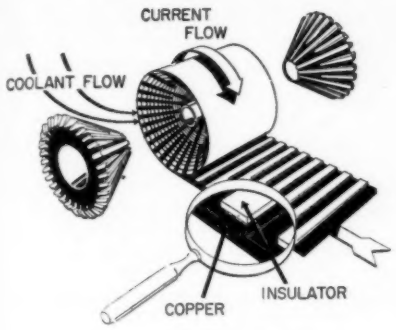

Fig. 1. Principle of the Kolm solenoid

ribs, and the ribs themselves consist largely of conductor with a minimum of insulation so as to reduce the loss of conductor volume and reduce the heat generation in uncooled portions of the tape. This structure provides such an enormous heat-transfer area that a helix of $\frac{1}{2}$-in. calibre, 3-in. outside diameter and 2-in. average length is capable in principle of dissipating the entire available power of 1·7 MW. In practice, it proved impossible to maintain adequate flow, and the first two solenoids failed at somewhat less than half the power. On the basis of this experience, it was then decided to build a solenoid of twice the size (Fig. 1). Since impedance match to the same generators must be maintained, tape thickness remains invariant under the scaling. This increases the number of necessary slots and ribs to such an extent as to make manual fabrication impractical. The third solenoid was therefore constructed by the High Voltage Engineering Corporation on a semi-automatic jig built especially for the purpose. After two unsuccessful attempts and several modifications in materials, technique and design, a solenoid was completed and installed in its housing (Fig. 2). It generated 126 kilogauss at a power consumption of 1·88 MW. The test occurred in mid-summer, when operation is limited by the high temperature of the primary cooling water from the Charles River; in winter it will probably be possible to increase the power sufficiently to generate more than 130 kilogauss.

The second phase of the programme, construction of a new laboratory, has also been making satisfactory progress. The original proposal was favourably received, and by the time funds were provided through the Air Force Office of Scientific Research, initial studies had been completed and the major decisions had been made. It will comprise essentially a power plant of four motor-generator sets capable of supplying 8 MW. of d.c. power continuously, 12 MW. for a 15-min. period, and up to 32 MW. for a 2-sec. period by the use of energy stored in flywheels. There will be ten cells or laboratories in which this power can be used, with space for expansion, and about 10^5 sq. ft. of area for supporting shops and laboratories. The generators will be separately controllable, so that four magnets can be operated simultaneously at 2 MW. each. The laboratory is expected to do for magnetic field research what laboratories like Brookhaven, for example, have done for nuclear physics. It will provide continuous fields up to about 250 kilogauss for visiting and resident scientists working in a number of different fields and also serve as a centre for advancing the art of magnet design. Construction of the laboratory is under the supervision of Prof. F. Bitter, and Dr. B. Lax will be its director. A broadly representative committee of prominent scientists is to guide all major policy decisions.

The third effort was first aimed at collecting and filing the sort of information on magnet design and performance data which are rarely if ever considered worth publishing. It began with a co-operative undertaking by a group of interested persons known as the "Magnet Society", and continues in the form of a punched card information file kept up by the staff of the new magnet laboratory. It is hoped that such information will ultimately be circulated in the form of periodical technical reports on magnet design and related subjects.

While working on the plans for the new laboratory, our group felt an increasing urge to discuss the project with a number of other scientists who might have occasion to use it. Since a new division of science, that of high magnetic-field research, was about to be born, it seemed logical to start things off with an international conference on the subject. The Air Force again generously agreed to provide sponsorship, and the date was set for November 1–4, 1961. Originally it was felt that this might coincide with the opening of the new laboratory, but as things turned out it will more nearly commemorate the ground-breaking. Papers to be presented at the conference will deal with the generation of high magnetic fields, as well as their use in plasma (ionized gas) physics, nuclear physics, solid-state physics and biology. Biological effects of magnetic fields have only recently attracted much attention, and the conference will provide the first opportunity for workers in this field to meet.

One cannot help but recall a similar conference in the field of low-temperature physics held in the Massachusetts Institute in 1949. It was originally intended to celebrate a great contribution to the field, namely, the invention of Prof. Co..ins's helium liquefier, but has since become a biennial event of great value. We

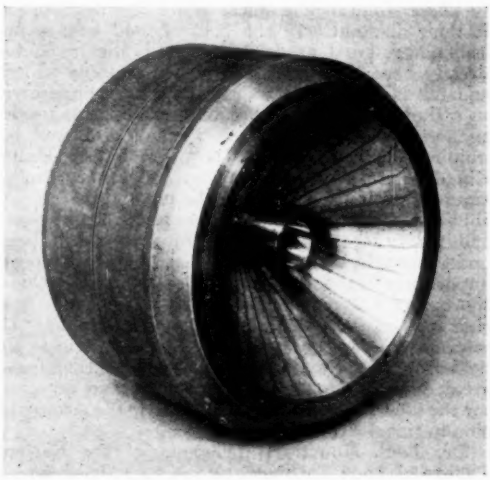

Fig. 2. Core of the new solenoid which generates 126 kilogauss at a power consumption of 1·88 MW. Calibre of the barrel is 1 in.; outside diameter of the tape winding is 6 in. 300 gallons per min. of cooling water are pumped through the small channels which are aligned like the spokes of a wheel

sincerely hope that the high-magnetic field conference is destined to comparable success. Like most divisions of science based on experimental difficulty, both cryogenics and magnetogenics are probably doomed to ultimate absorption into the more legitimate disciplines. As in the case of cryogenics, however, high-magnetic field research will first experience a golden age of contributing a great many stimulating problems.

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TRAINING OF INDUSTRIAL FOOD SCIENTISTS AND TECHNOLOGISTS

THE "Training of Industrial Food Scientists and Technologists in the United Kingdom" was discussed on September 5 by Section B (Chemistry) of the British Association for the Advancement of Science, meeting at Norwich.

Britain's war-time food problems drew sharp attention to the practical importance of the application of scientific knowledge to the processing and distribution of food. Likewise difficulties in obtaining technologists skilled in this field of work became apparent, and to meet this need the then Ministry of Food set up a working party in 1948 to study means of training. A direct result of these endeavours was the establishment of the National College of Food Technology in London and the introduction of courses in food science at the Royal College of Science and Technology in Glasgow. Although these centres have flourished, it has recently become clear that their joint efforts are insufficient to meet the rising demand. This discussion at the British Association was therefore particularly appropriate in timing and content.

Sir Herbert Broadley (U.N. International Children's Emergency Fund) dealt with international aspects of the problem in his opening paper. His theme, which was to be emphasized by subsequent speakers, was the opportunity open to the United Kingdom to assist the under-developed countries in a realistic way by placing our technological and scientific resources at their disposal. In the field of food science and technology, great needs exist, and while much has already been done, the shortage of suitable experts remains as a limiting factor which cannot be resolved on a short-term basis. Not only are experts needed in the field to tackle the most urgent problems, but training facilities are also required in the United Kingdom for overseas students. Only very limited numbers of overseas nationals have been able to obtain such training partly because of lack of places and partly because of the limited research being undertaken in Britain on food problems of special importance to the under-developed countries. One of the major malnutrition problems is associated with shortages of protein foodstuffs, and increasingly the international agencies are forced to seek help in this field from the United States. There is therefore a great opportunity for the universities and especially for the new universities of Britain to make a contribution in this field.

Dr. J. S. Mounfield (principal of the National College of Food Technology) analysed the difference in function of the food scientist and the food technologist and emphasized the importance of integration with industry in the training of the technologist. While it is true that the food technologist requires a rather less intensive knowledge than the food scientist of any single basic discipline such as chemistry, physics or biology, he must nevertheless be given some training in engineering and management to help him to solve the problems of large-scale processing.

Dr. Mounfield quoted a survey carried out about two years ago which suggested that the United Kingdom's needs for trained food scientists and technologists were between 150 and 250 per annum. Compared with these numbers, the present output is very small. In these circumstances, it is difficult to see how we can make more than a token contribution to the training of students from under-developed countries. Furthermore, it is clear from applications received at the National College that inadequate basic scientific training in some countries denies students the opportunity to profit from facilities which can be offered here. He proposed that special qualifying courses be organized in the United Kingdom to enable such students to reach the required entrance standards. The admissions problem was complex, and to increase the effectiveness of existing facilities for study he recommended the formation of a committee to foster co-operative effort between the research associations, the food industry and the education authorities.

Prof. J. Hawthorn (professor of food science, Royal College of Science and Technology, Glasgow) began by considering the educational principles governing the training of food scientists as opposed to food technologists and emphasized the importance of the basic disciplines of physics, chemistry, biochemistry and microbiology in the development of the student's thought. He reported the first stage of a survey being carried out by his own department on opportunities for graduates in food science, and estimated the apparent annual demand of the United Kingdom at between 150 and 250 per annum, thus agreeing with Dr. Mounfield's estimate, which had been obtained independently. This apparent demand, however, is almost certainly less than the real need and even the apparent demand is far in excess of the numbers of graduates actually produced. He then pointed out that the Universities of Nottingham and Reading have now followed the example of Glasgow and would introduce degree studies in this subject as from 1961. The University of Leeds would follow in 1962.

Dr. J. W. Corran (chief chemist of J. and J. Colman, Ltd.) then opened the discussion by making a formal proposal to the meeting that it should recommend to the Council of the British Association that an *ad hoc* committee be formed to consider

existing schemes for training food scientists and technologists, to suggest means of extending the available facilities, to consider whether the food research associations could contribute to such training schemes and to bring any recommendations to the notice of the appropriate overseas departments of the Government. He also suggested that the membership of such a committee should include both scientists associated with food science and also persons with experience of conditions overseas and in under-developed countries. After discussion, the proposal was adopted.

Perhaps the main impression left in the minds of those attending was the growing sense of urgency in this field of work, and the feeling that the United Kingdom could and should do more to assist the under-developed countries in their drive to raise the nutritional standards of their peoples. While it was comparatively easy to discuss training needs and facilities, real progress would depend on the work of the professional teachers who would be concerned with those essential but tiresome details of entrance qualifications, standards and course structures. The root of the difficulties lay not merely in the availability of money to be spent in developing such facilities but also in the differences in educational standards between the under-developed countries and the United Kingdom. J. HAWTHORN

INTERACTION OF WEST FORD NEEDLES WITH EARTH'S MAGNETOSPHERE

By PROF. S. F. SINGER
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W. E. MORROW[1], of the Lincoln Laboratory of the Massachusetts Institute of Technology, has suggested the use of a new method for intercontinental communication. It is based on the use of belts of orbiting dipoles which act as a scattering medium for microwaves. This technique could have significant advantages over other satellite communication techniques. However, it has been pointed out by others, particularly observational astronomers, that this operational project labelled West Ford would have some undesirable side-effects on optical astronomy[2] and on radio astronomy[3]. This article is not concerned with these effects nor with communications and related topics[4]. Instead I shall examine in some detail certain aspects of the interaction of the West Ford medium with the exosphere, in particular the interaction of the electric charge, which the needles are certain to carry, with the ionized portion of the exosphere. I wish to point out the possibility of making unique observations which can help establish the important parameters of this interaction. Therefore, so far as possible, this article will give numerical predictions of orbit changes. In addition, a useful modification of the experiment is suggested.

Forces acting on Small Particles in Near Space

The unique properties of the orbiting dipoles arise from the fact that they are so small. They have, in fact, close to the largest area-to-mass ratio (50 cm.²/gm.) of any artificial satellite, and approach an area-to-mass ratio of small ($\sim 100\mu$) interplanetary dust particles. Hence we can apply to the West Ford medium an analysis used for interplanetary dust in the vicinity of Earth[5]. [See also p. 321 of this issue.]

The motion of a particle is determined by the forces which act on it. Within the region of Earth's exosphere (taken to extend out to about 10 Earth radii) the gravitational force of Earth predominates and will be orders of magnitude greater than other forces, even for small particles. However, there are three other forces which need to be taken into account, particularly for orbiting particles: (1) radiation pressure; (2) the Lorentz force produced by Earth's magnetic field; (3) Coulomb force produced by the interaction with the ionized exospheric gas.

(1) The radiation pressure force is chiefly due to solar radiation and, to a smaller extent, to the radiation scattered from and emitted by Earth. The dynamic effects of the radiation pressure force have been investigated by Jones and Shapiro[6]. The effects predicted for the West Ford medium are given in a paper by Morrow and MacLellan[4] and would indicate that the medium disperses at a certain rate; also that the change in eccentricity produced by a resonant phenomenon would be particularly strong for polar orbits at altitudes of 3,700 km.

(2) The Lorentz force acts perpendicular to the velocity vector of the particle. It will be most pronounced in the polar regions where the magnetic field is strong and always perpendicular to the velocity vector of the particle. Only one general statement may be made about the effects of the Lorentz force: it cannot change the total energy of the particle. In other words, the semi-major axis of the orbit will remain constant, but the other parameters of the orbit may vary.

(3) The Coulomb (electrostatic) drag force has been shown to be one of the most important forces for particles moving in interplanetary space[7]. This electrostatic drag force acts generally between any charged body moving through a plasma and has therefore been also considered for the case of Earth satellites by various authors who, however, arrive at varying conclusions[8–13]. My view agrees most closely with that of Lehnert[8], who first showed that the Coulomb drag would not be particularly effective for Earth satellites of nominal dimensions.

The importance of the Coulomb force then hinges on two points: (*a*) the small size of the body; (*b*) the question of its charge. In general we may say that if the body were very much smaller than the Debye screening distance, then the question of the Coulomb drag should be investigated in detail. The dynamics of the charging situation was investigated by me, and I also discussed the competition between photoelectric emission and accretion of charge by ambipolar diffusion from the surrounding plasma. It was concluded that in interplanetary space dust particles would most likely be positively charged[7]. Whipple[14] has pointed out explicitly that the charge becomes less positive

as one approaches Earth, and becomes negative in the ionosphere.

Since the Coulomb drag produces a dissipation effect akin to friction, it will decrease the semi-major axis of the orbit continuously and eventually cause the particle to enter the dense atmosphere of Earth. I am therefore particularly interested in investigating the effect of Coulomb drag on the particle's life-time.

Analysis of the Coulomb Drag Force

For the purposes of discussion I shall adopt a treatment introduced by Chandrasekhar and Spitzer in which the orbiting particle will be referred to as the 'test particle' and the ions and electrons of the exosphere as the 'field particles'. The drag deceleration is given by[15]:

$$a_C = - A_D \left(\frac{m_1}{2kT_1}\right)\left(1 + \frac{m}{m_1}\right) G(x) \qquad (1)$$

where the subscript 1 always refers to the field particles;

$$x = (1{\cdot}5)^{1{\cdot}5}\, w/C_1$$

where $C_1 = (3kT_1/m_1)^{1/2}$, and is the root mean square velocity, and w is the test particle velocity ($\sim$ 5 km./sec.).

$$G(x) = \frac{\varphi(x) - x\,\varphi'(x)}{2x^2}$$

$$\varphi(x) = 2\pi^{-1/2}\int_0^x \exp(-y^2)\,dy$$

$$A_D = 8\pi e^4 n_1 Z_1^2 Z^2\,(\ln\Lambda)/m^2$$

$$\Lambda = 3(2Z\,Z_1\,e^3)^{-1}\,(k^3T_1^3/\pi\,n_1)^{1/2}$$

where n and Z denote particle concentration and charge, respectively, for the exosphere $Z_1 = 1$.

With an exospheric temperature T_1 of 1,500° K., we obtain: $C_1 = 6{\cdot}1$ km./sec. for protons; $C_1 = 260$ km./sec. for electrons. Under these conditions $\ln\Lambda \sim 20$; $G(x) \sim 0{\cdot}2$ for the field protons and less than 0·015 for field electrons. Since the particle mass $m \gg m_1$, equation (1) reduces to:

$$a_C = 1{\cdot}23 \times 10^{-23}\, Z^2\, n_1/m \qquad (2)$$

In the derivation of equation (2) some approximations have been made; for example, the particle's velocity w was assumed constant and appropriate to a circular orbit at an altitude of 9,500 km. No greater refinement appears necessary in view of the gross approximations which must be made in the calculation of the charge Z.

Calculation of Electric Charge

The electric charge of the particle is given by its electric capacity multiplied by the potential of its surface. For a conducting sphere of radius a cm.:

$$Z = 6{\cdot}95 \times 10^6\, aV \qquad (3)$$

where V is measured in volts. For a body of different shape the capacity must be calculated; in the absence of detailed data we will adopt a system of two spheres each with 'effective' radius of $1{\cdot}5 \times 10^{-3}$ cm. Thus for the West Ford needles, we take:

$$Z \sim 2 \times 10^4 \text{ V.} \qquad (4)$$

The calculation of the potential V also presents special problems since it depends on the position of the particle, and therefore in turn on the characteristics of the particle's orbit. It also depends on the work function W and photoelectric yield γ of the particle material, its shape and its attitude towards the Sun. As explained earlier, we calculate V in an analysis involving competition between the photoelectric effect and ambipolar diffusion[7]. The ambient density above $\sim 1{\cdot}5\ R_E$ (Earth radii) is given by Helliwell as:

$$n_1 \sim 10^4\, r^{-3} \qquad (5)$$

where r is measured in terms of Earth radii.

The photo-emission is determined by the ultraviolet spectrum of the Sun; that is, by N_ν (the photon flux per cm.²/sec. with energy $h\nu \geqslant eV + W$). I assumed the relation $N_\nu = fV^{-\delta}$; where $f = 10^{13}$ and $\delta = 4$. On the basis of Hinteregger's measurements[16], a better set of values is:

$$N_\nu = 2{\cdot}2 \times 10^{13}\, V^{-4.444}\ [\text{cm.}^2\text{sec.}]^{-1} \qquad (6)$$

The equilibrium potential is given by ref. 10:

$$V_+ \sim + (\gamma f/n_e C_e)^{1/\delta} \qquad (7)$$

when the particle has a large positive potential and the ion current can be neglected. In Earth's shadow, where photo-emission is negligible, the equilibrium potential is given by:

$$V = - (kT_e/2e)\,\ln\,(m_i T_e/m_e T_i) \qquad (8)$$

In view of the uncertainties in γ, W, attitude and orbit orientation, I have decided to make some drastic approximations and represent the potential by:

$$V = 7{\cdot}5\,(r - 2) \qquad (9)$$

As can be seen, V varies from $-7{\cdot}5$ V. in the F-layer to + 60 V. at 10 R_E. Note that $V = 0$ at 2 R_E; the implication will be discussed later.

It should perhaps be pointed out explicitly that another powerful charging process exists, namely, the high flux of fast electrons in the outer radiation belt; it is, however, quite localized and confined mainly to the vicinity of the lines of force which extend to 3–5 R_E in the plane of the magnetic equator. Its effect is to produce (or enhance) the negative charge (by accretion and secondary electron emission); this effect must be considered for orbits which intersect the outer radiation belt.

By combining equations (2), (4), (5) and (9) we obtain for the Coulomb drag deceleration:

$$a_C \sim 3 \times 10^{-9}\,(r-2)^2\, r^{-3}/m$$

With the needle mass given[4] as 10^{-4} gm.:

$$a_C \sim 3 \times 10^{-5}\,(r-2)^2\, r^{-3}\ [\text{cm./sec.}^2] \qquad (10)$$

This acceleration may be compared with the accelerations due to:

(i) Radiation pressure:

$$a_{RP} \sim \left(\frac{\text{solar constant}}{\text{speed of light}}\right)\left(\frac{50 \text{ cm.}^2}{\text{gm.}}\right) = 2{\cdot}5 \times 10^{-3} \text{ cm./sec.}^2 \qquad (11)$$

(ii) Gravity:

$$a_G = 980\, r^{-2} \qquad (12)$$

(iii) Magnetic:

With the circular velocity $w \sim 8 \times 10^5\, r^{-1/2}$ cm./sec., and with the magnetic field $B \sim 0{\cdot}5\, r^{-3}$ gauss, we obtain:

$$a_M = Ze\,w\,B/m = 10^{-5}\,(r-2)\,r^{-3.5} \text{ cm./sec.}^2 \qquad (13)$$

Effects of Coulomb Drag

The changes in orbit and life-time of a satellite in the presence of a dissipative drag force F_D can be treated most simply by energy considerations[17].

The loss of energy per orbit in a circular orbit is $\Delta E = 2\pi r F_D$ and this will reflect itself in a lowering of the orbit by an amount Δr in a time T which is simply the orbital period:

$$T = 2\pi\,(GM_E)^{-1/2}\,r^{3/2} \qquad (14)$$

Since the total (kinetic + potential) energy is given by $E = \frac{1}{2}\,GM_E m/r$, we obtain by differentiation:

$$\Delta r = (-\Delta E)\;2r^2/GMm \qquad (15)$$

We can therefore obtain an expression for the rate of shrinkage of a circular orbit as follows:

$$\Delta r/\Delta t = 2\pi r F_D \cdot \frac{2r^2}{GMm}\,\frac{(GM)^{1/2}}{2\pi r^{3/2}} =$$

$$\frac{2}{(GM)^{1/2}}\,\frac{F_D}{m}\cdot r^{3/2} = \frac{1}{\pi}\,T\,\frac{F_D}{m} \qquad (16)$$

which is $(1/\pi)$ times (the orbital period) times (drag deceleration).

We can also express $(\Delta r/\Delta t)$ as a function of r using equation (10) for Coulomb drag and using $T = 5{,}080\,r^{3/2}$ for orbital period :

$$(\Delta r/\Delta t)_C = \sim 5 \times 10^{-3}\,(r-2)^2\,r^{-3/2}\ \text{[cm./sec.]} \qquad (17)$$

This expression may now be compared with the neutral drag acceleration evaluated from:

$$F_N/m = \tfrac{1}{2}\,C_D A\rho\,w^2/m =$$

$$\tfrac{1}{2}\,C_D\,A(n_H m_H + n_0 m_0)\,w^2/m \qquad (18)$$

where C_D is the drag coefficient, A the projected area and ρ the density measured in gm./cm.$^{-3}$.

Using $w^2 = (7{\cdot}9\times10^5)^2/r$; $m = 10^{-4}$ gm.; $A = 5\times10^{-3}$ cm.^{2}; $C_D \sim 2$; we obtain:

$$F_N/m = 5\times10^{-3}\times1{\cdot}66\times$$
$$10^{-24}\,(n_H + 16\,n_0)\,(6{\cdot}25\times10^{11})\,10^4/r$$
$$= 5{\cdot}2\times10^{-11}\,(n_H + 16\,n_0)/r\ \text{[cm./sec.}^2\text{]} \qquad (18')$$

In the case of a particle belt, it is more relevant to calculate $(\Delta r/\Delta t)$ rather than the change in orbital period:

$$(\Delta r/\Delta t)_N = 1/\pi\,(5{,}080\,r^{3/2})\,5{\cdot}2\times10^{-11}\,(n_H + 16n_0)/r$$
$$= 8{\cdot}4\times10^{-8}\,(n_H + 16\,n_0)\,r^{1/2}\ \text{[cm./sec.]} \qquad (19)$$

It is of considerable interest to establish the relative importance of the two drag forces. In order to do this we must adopt a model for the structure of the magnetosphere.

Observational information is limited to rather low altitudes. The distribution of mass density below 800 km. is known from analysis of satellite drag[18]. The integrated thickness of the neutral hydrogen cloud around Earth is known from analysis of the profile of solar hydrogen Lyman-α (ref. 19). Electron densities up to 1,500 km. are known from rocket measurements[20]. The electron density to several Earth radii is inferred (with some uncertainty) from whistler observations[21,22].

On the other hand, the *relative* distributions of the neutral constituents can be deduced from a theory of the exosphere[23]. The neutral components describe ballistic orbits without collisions above the base of the exosphere (530 km.) and the distribution of concentration with altitude is calculable in a fairly straightforward manner. The ionized components (having much larger cross-section because of Coulomb interaction) are distributed according to the barometric formula[24]. In each case the slope (or scale height) is determined by the temperature at the base of the exosphere. Using the data referred to above for normalization, it is possible to construct a model of the exosphere[25]. This model is shown in Table 1.

Table 1. Concentration of Major Constituents in the Terrestrial Exosphere (cm.$^{-3}$)

r/R	O	O$^+$	H	H$^+$
1·100	1·2 × 10^7	5 × 10^5	10 × 10^3	—
1·200	2·7 × 10^6	2·5 × 10^4	6·6 × 10^3	9 × 10^3
1·300	1·7 × 10^5	1·7 × 10^3	4·4 × 10^3	6·6 × 10^3
1·400	1	1·3 × 10^3	3·1 × 10^3	5 × 10^3
1·500	—	11	2·3 × 10^3	3·7 × 10^3
1·75		< 0·1	1·25 × 10^3	2·1 × 10^3
2·00			8 × 10^2	1·3 × 10^3
3·00			2 × 10^2	3·5 × 10^2
4·00			82	1·5 × 10^2
5·00			43	85
6·00			25	51
7·00			16	33
8·00			12	23
9·00			9·1	17
10			6·5	11

Both neutral and Coulomb drag decelerations are shown in Table 2, as well as the resulting values of $(\Delta r/\Delta t)$. It must be pointed out, however, that the Coulomb drag depends on many detailed factors (some of which were discussed earlier), involving the particular properties of the particle, of the exosphere, and of the Sun, as well as the particulars of the orbit.

The zero value of Coulomb drag at $2R_E$ should not be taken literally; it merely indicates that somewhere in this altitude-range there may be a minimum. As discussed elsewhere, small fluctuations of charge will produce a drag (since it depends on the square of Z and not on the sign); in particular, the day–night effect sets a lower limit to the Coulomb drag[5]. This minimum may give rise to a high concentration of interplanetary smoke (particles $\lesssim 1\mu$) near 2 Earth radii if they exist.

Discussion

It would be of interest to calculate the life-times of the West Ford particles. Since the details of the orbit have not been revealed, and, in particular, since the altitude and orbit inclination are not known, it does not seem worth while to attempt any refined calculations. There are in any event uncertainties about the electric charge of the particle, about its attitude with respect to magnetic field and with respect to the Sun, and also about the actual values of air density.

With initial orbit details available, the subsequent history of the West Ford particles can only be obtained by numerical methods. It is clear, by referring to Table 2, where the neutral drag may be neglected. It is clear also, by referring to equations (10), (11), and (13), that probably none of the other forces can be neglected, except for very special orbits. (It is interesting to note the importance of the radiation pressure in relation to the Coulomb drag. This is due to the geometric shape of the needles. It should be noted that for a spherical interplanetary dust particle of the same mass, the radiation pressure acceleration is $\sim 5\times10^{-5}\,\pi a^2/m$ and is, relatively,

Table 2. Drag Deceleration and Orbit Shrinkage

r/R	a_N (cm./sec.^{2})	$(\Delta r/\Delta t)_N$ (cm./sec.)	a_e (cm./sec.^{2})	$(\Delta r/\Delta t)_C$ (cm./sec.)
1·1	9 × 10^{-3}	1·7 × 10	1·8 × 10^{-5}	3·5 × 10^{-2}
1·2	3·6 × 10^{-5}	7·6 × 10^{-2}	1·1 × 10^{-5}	2·4 × 10^{-2}
1·3	1·5 × 10^{-6}	3·6 × 10^{-3}	6·9 × 10^{-6}	1·7 × 10^{-2}
1·4	3·7 × 10^{-7}	1 × 10^{-3}	4·0 × 10^{-6}	1·1 × 10^{-2}
1·5	2·1 × 10^{-7}	6·1 × 10^{-4}	2·2 × 10^{-6}	6·8 × 10^{-3}
1·75	9·8 × 10^{-8}	3·7 × 10^{-4}	3·6 × 10^{-7}	1·4 × 10^{-3}
2·0	5·5 × 10^{-8}	2·5 × 10^{-4}	(0)	(0)
3·0	9·5 × 10^{-9}	7·9 × 10^{-5}	1·1 × 10^{-6}	9·6 × 10^{-3}
4·0	3 × 10^{-9}	3·9 × 10^{-5}	1·9 × 10^{-6}	2·5 × 10^{-2}
5·0	1·4 × 10^{-9}	2·4 × 10^{-5}	2·1 × 10^{-6}	4·0 × 10^{-2}

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100–1,000 times smaller when compared with the Coulomb drag[5,7].)

In a numerical computation, particular attention must be paid to the day–night effect, not only on radiation pressure, but also on the particle charge. In fact, because of fluctuations in radiation belt intensity, solar flares, and similar factors, the charge will fluctuate unpredictably. This effect will contribute to a spreading of the belt which is, in addition to the dispersion, calculated by the Lincoln Laboratory[4].

Nevertheless, the shrinkage of an initially circular orbit produced by loss of energy due to drag is a gross effect which should be observable, and which gives some information on air density as well as about the importance of Coulomb drag.

A Proposed Experiment

However, a much more refined experiment is possible which illuminates the interaction of small particles with the magnetosphere. It is based on a separation of the perturbing forces.

Consider, for example, an initially circular orbit at ~ 3,200 km. altitude ($r = 1{\cdot}5\ R_E$), nearly polar and perpendicular to the Earth–Sun line. In this special orbit the radiation pressure does not produce any appreciable perturbation (at least initially), and the Lorentz force perturbation can be determined quite simply, although it is again negligibly small. Since the velocity vector of the particle is more or less in the plane of the magnetic meridian, the chief perturbation will be orthogonal and cause a rotation of the orbital plane. The sense of the rotation establishes the sign of the electric charge on the particle. Using the analysis leading up to equation (13) I estimate the rate of rotation of the orbital plane as about 10^{-11} rad./sec., and rather unobservable.

Next consider a modification of the photoelectric properties of the copper needles. Specifically, one-third could be left with their present copper surface, one-third would be coated with a material of high work function W and low photoelectric yield γ (for example, platinum), and the remainder with a material of opposite characteristics (possibly a cæsium alloy). For our particular orbit, the copper particles would be negative, the platinum particles even more negative, while the cæsium particles would have a nearly zero potential, or perhaps be even positive.

Because of Coulomb drag the three belts will shrink at different rates. Using the analysis leading up to equation (17) I estimate a differential rate of shrinkage of orbit radius amounting to nearly 5 m. a day.

Thus the separation of this West Ford belt into three layers might be resolvable with available radar methods, provided the dispersion of the particles at injection can be held very small.

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NEWS and VIEWS

Royal Aircraft Establishment: Mr. R. J. Atkinson

Mr. Ronald John Atkinson has been promoted to deputy chief scientific officer and has taken up the post of head of the Structures Department at the Royal Aircraft Establishment, Farnborough, in succession to Dr. Percy B. Walker, who has been appointed special consultant to the director of the Establishment. Mr. Atkinson, an Australian, came to Great Britain in 1936 with a Rhodes Scholarship from the University of Queensland. After studying at Oxford under Sir Richard Southwell, he went to the Imperial College of Science and Technology for a year; and later was with Fairey Aviation, Ltd., at Stockport. He joined the Royal Aircraft Establishment in May 1940, and was appointed head of the Fatigue Division of the Structures Department in 1955. He is a bachelor of engineering of the University of Queensland, holds the diploma of the Imperial College and is a Fellow of the Royal Aeronautical Society.

Research and Development in the Gas Industry

Dr. J. A. Gray, group leader of physical chemistry and chemical engineering at the Battelle Memorial Institute, Geneva, since 1958, has been appointed head of the new Basic Research Group which the Gas Council is establishing at its London Research Station at Fulham. Dr. Gray is a graduate of King's College, University of London, and prior to joining the staff of the Battelle Memorial Institute, was employed in the Alkali Division of Imperial Chemical Industries, Ltd. In forming the Basic Research Group, the Gas Council's intention is to provide the industry with the means of developing entirely new ideas for the production and utilization of gas without being bound by short-term considerations, or the need to produce results to a definite programme. Although, for administration purposes, it will form part of the Council's London Research Station, the Basic Research Group will operate independently, and the head of the Group will be a member of the

Council's Research Committee, and will exercise full control over the Group's scientific work.

Air Force Cambridge Research Laboratories

MAJOR changes were recently announced for the Air Force Cambridge Research Laboratories, Bedford, Massachusetts, by the commander, Brigadier-General B. G. Holzman. The changes were aimed at strengthening the research programme and the management structure of the Laboratories, and preventing the tendency for overlap of work within the Directorates. The separate staffs of the two Directorates of the Air Force Cambridge Research Laboratories, the Electronics Research Directorate and the Geophysics Research Directorate, plus a small headquarters staff, were combined to form a single headquarters staff that now serves both Directorates. However, the various laboratories coming within these Directorates are not affected by the reorganization. Heads of the four major staff elements are: Dr. A. M. Gerlach, deputy for plans and programmes; Lt.-Colonel Eugen Duff, deputy for administrative management; Mr. V. S. Dempsey, deputy for logistics; and Mr. L. E. Woods, research information officer. In addition, a new division was created to provide technical services to the Laboratories' scientists under the direction of Mr. V. S. Dempsey. Dr. L. M. Hollingsworth continues as director of electronics research. Colonel Ernest A. Pinson continues in the dual capacity of the Laboratories' deputy commander and director of geophysics research, positions he has held for the past year. However, Colonel Edward M. Douthett, at present assigned to Headquarters, U.S. Armed Forces, will assume duty as director of geophysics research in mid-November.

Revision of School Science Curricula

IN reply to a question from Viscount Samuel in the House of Lords on October 18 as to what action was being taken to implement the recommendation of the Advisory Council on Scientific Policy regarding the revision of school science curricula, the Minister for Science, Lord Hailsham, said that this has been carefully considered by himself and the Minister of Education. In England and Wales the initiative for framing curricula and syllabuses rests with the teaching profession and, after a very thorough review, the Association of Women Science Teachers and the Science Masters' Association have recently proposed substantial revisions and modernization of existing syllabuses. The Minister of Education is encouraging these and similar efforts to revise the syllabuses, and the proposals of the Science Masters' Association are being used by the General Certificate of Education examining bodies as the basis for the revision of their examination syllabuses which they are undertaking at the request of the Secondary School Examinations Council. This Council advises the Minister on the general direction of the General Certificate of Education examination, and schools, universities, local education authorities and other bodies concerned are represented on it. Corresponding action has been taken independently in Scotland through the Scottish Education Department.

The CERN Laboratory, Geneva

IT was at the end of 1959 that the 25-GeV. proton synchrotron came into operation in the CERN laboratory at Geneva (European Organization for Nuclear Research, CERN. Annual Report, 1960. Pp. 113. Geneva: CERN, 1961). During 1960 the beam intensity was increased ten-fold to 10^{11} protons/sec. and the experimental hall was equipped for beam transport. In the past quarter 504 operating hours were devoted to nuclear physics experiments. These experiments opened up a new energy region in the study of the elementary particles. On the synchro-cyclotron the experimental groups have continued to make distinguished contributions to meson physics. Probably the most impressive of these was the measurement of g-2 for the muon to an accuracy of 2 per cent. The completion of the new muon channel must mean major progress in muon physics within the next few years. To cope with the increasing amount of experimental data, particularly from bubble chambers, an IBM 709 computer was installed during the year.

The original programme defined in the convention of 1953 is now virtually complete. This is a brilliant achievement but, as the report makes clear, CERN has no intention of resting on its laurels. Work is in progress to improve the facilities on both the accelerators and a major contribution to nuclear physics is guaranteed for many years to come. Even so, the director-general, in his introduction, refers to longer-term plans for a new accelerator in 1970. All this is an indication that the CERN laboratory is a lively and interesting place, an outstanding example of international collaboration. Confirmation of this can be found by reading the report, which gives a vivid and particularly well-illustrated account of the laboratory's achievements and activities in 1960.

Political and Economic Planning

THE annual report of Political and Economic Planning for the year ended March 31, 1961, includes a list of publications, 1960–61, and of PEP staff for the year, but deals essentially with the research programme. Continued progress is reported with the study of Britain and the European Common Market and the preparation of a report on Atlantic Tariff and Trade has proved to be a major undertaking. This study is assisted with a generous three-year grant from the Ford Foundation. Two new major projects launched during the year were concerned with trade unions in a changing society and with present-day trends in the development of community mental health services, the former being supported by a substantial grant from the Leverhulme Trust Fund, and the latter by a grant from the Nuffield Provincial Hospitals Trust. An inquiry is also being launched into the effects on wives and dependants when the chief male earner is imprisoned. An examination of the work of professional and learned societies, with special reference to any difficulties imposed by modern industrial conditions, is also being carried out, and provisional help has been promised by the Department of Scientific and Industrial Research.

Scientific Research in Australia

THE Research Review of the Commonwealth of Australia Scientific and Industrial Research Organization for the year ending June 30, 1960, is the complement of the shortened form in which the Organization's twelfth annual report was presented, with which it is intended to be read (Pp. iv+418. Melbourne: Commonwealth Scientific and Industrial Research Organization, 1961). It presents the scientific details of the research programme in much the same way as the earlier annual reports, each of its 48 chapters being devoted broadly to one division or

section of the Organization. Lists of professional staff, published scientific papers and financial details are excluded and are given in the annual report.

Research Grants in Science and Medicine in Canada

THE report on *University Support*, 1960–61 (Pp. 123. N.R.C. No. 6390. Ottawa: National Research Council and Medical Research Council, 1961. 50 cents), issued by the National Research Council and the Medical Research Council of Canada, records support totalling 8,169,564 dollars from the National Research Council and 2,304,467 dollars from the Medical Research Council. Of the former figure, 7,169,402 dollars represent direct support by way of scholarships in science and engineering (738,467), fellowships in science and engineering (396,758) and grants in aid of research through the Assisted Researches Committee (4,267,195), Prairie Regional Committee (232,458), Atomic Energy Control Board (650,000) or General Research grants (492,000), and 1,000,162 dollars indirect support, including 285,589 dollars for publication costs of research journals and 227,334 dollars for the administration of the programme. The latter includes 189,326 dollars for fellowships, 204,191 dollars for associateships and 1,899,809 dollars for grants in aid of research. The totals represent increases of 1·11 million dollars over 1959–60 and 7 million dollars over 1956–57. Besides listing the membership of the Councils and their officers and committees, the report lists the recipients of fellowships, bursaries and scholarships for the period 1960–61 and the subjects of research grants.

The Wildfowl Trust

THE Wildfowl Trust has again prepared an annual report which is a model of presentation (*The Twelfth Annual Report of the Wildfowl Trust, 1959–1960*. By Hugh Boyd and Peter Scott. Pp. 185+24 plates. Slimbridge: The Wildfowl Trust, 1961. 17*s*. 6*d*. net). Practical administrative matters are described clearly and briefly, scientific topics are many and varied, and there are the usual high-quality photographs and line drawings which make the reading of the report an event to be anticipated rather than a duty to be overcome. Unfortunately, the twelfth year of the life of the Trust has seen a drop in the number of visitors to Slimbridge and Peakirk, and it is to be hoped that the decline is only temporary. Probably the most interesting and disturbing scientific article is one by J. M. and J. G. Harrison, who have collected evidence to show that radioactive contamination in birds is beginning to increase. While risks to wildfowl are but a tiny facet of the bigger problem of radioactive contamination, wildfowl may be particularly susceptible, especially those breeding in the far North, where the hazards from radioactive fall-out may be at their greatest. The added risks from the disposal of radioactive industrial waste products may again render wildfowl and seabirds more susceptible than other groups of birds.

London and Guildhall Museums

THE Government and the Corporation of London and the London County Council have agreed in principle to establish one Museum dealing with the history of London. This will involve the amalgamation of the present London and Guildhall Museums and the full approval of each of the governing bodies is assured. The new Museum will be situated in the City of London, probably on a site close to the Roman Wall. Although details have not yet been agreed, it is probable that the governing body will consist of representatives appointed by the Prime Minister, the City Corporation and the London County Council. When the details have been agreed by the authorities, an Act of Parliament will be necessary to implement the scheme.

Rocket Propulsion Thirty Years Ago

THE science and technology of rockets have advanced from being the hobby of a few apparent cranks to become major consumers of taxpayers' money; even in the universities the type of student who immediately after the War would have sought to accomplish research on the gas turbine, and later would have followed changing fashion and interested himself in nucleonics, now regards rockets as pointing the way to the stars. In spite of the great achievements of the age, however, it is interesting to look back at the pioneer work of another student at the Imperial College of Science and Technology, London. A paper, originally read by Mr. Paul Adorian in 1929, has been reprinted and the proceeds from the sale of these reprints is to be given to the Henry Tizard Memorial Fund. The paper contains little original matter, but it does give a picture of the approach to rocket propulsion more than thirty years ago, and a contemporary point of view of such developments as the Opel rocket cars, designs for jet aircraft curiously modern in style, and a German rocket gun which its open-minded inventor claimed could "shoot London from Berlin, or if you prefer it, shoot Berlin from London". Copies of the reprints are available from Mr. P. Adorian, 21 Denmark Street, London, W.C.2 (price 8*s*. or 1.25 dollars).

The Cambridge Rheology Club

A RECENT meeting of the Cambridge Rheology Club was held on September 11 in the form of an exhibition of rheological equipment. Mr. C. H. Rose (Cambridge Instrument Co.) demonstrated the dye dilution curve recorder and described its uses in the investigation of certain defects of the vascular system. Grant Instruments showed notably a low-temperature thermostatically controlled bath (Model *RB6P*). Miss M. Nicholas (Spillers, Ltd.) demonstrated the cattle cube hardness tester, which may also be used for strength tests on paper and similar materials. She dealt briefly with the statistical evaluation of the results. Dr. N. A. de Bruyne (Techne, Ltd.) discussed an extensive range of rheological instruments, including the gelation timer, capillary viscometer, various temperature control units and the automatic printing viscometer, still in the experimental stage. The next meeting of the Club will be held on November 27, at 7.45 p.m., when Dr. R. McKennell (Ferranti, Ltd.) will speak on "Rotation Viscometers and their Application to Non-Newtonian Measurements", followed by a demonstration of the Ferranti–Shirley viscometer. Further information can be obtained from Mr. H. G. Muller, Spillers, Ltd., Technological Research Station, Station Road, Cambridge.

Tables and Charts for Use in Gas Dynamic Studies

THE Sandia Corporation has recently issued a very useful bibliography (*SCR*–299), by D. E. Randall, listing references which provide tabulated or graphical data regarding the atmosphere, gas flows, flow through shock waves, and information on the thermodynamic and transport properties of gases. Included in the list is a new Sandia monograph (*SCR*–288), by K. L.

Goin, entitled "Mach Tables for Real Gas Equilibrium Flow of Air in Hypervelocity Test Facilities with Total Temperatures to 10,000° K.". This report tabulates the aerodynamic flow parameters for isentropic expansions and flow through normal shocks in the pressure range 100–4,000 atmospheres. Both the bibliography and the monograph are available, price 0·75 and 2·0 dollars, respectively, from the Office of Technical Services, Department of Commerce, Washington 25, D.C.

Gairdner Foundation Awards

GAIRDNER FOUNDATION awards have been made to the following in recognition of their contributions to the study of arthritis and heart diseases: Dr. Alan C. Burton, professor of biophysics, University of Western Ontario, for his work on blood circulation; Sir Russell Brock, cardiac surgeon at Guy's Hospital and Brompton Hospital, London, for his work on heart surgery; Dr. Jonas H. Kellgren, professor of rheumatology and director of the Rheumatism Research Centre, University of Manchester, for his work on the incidence of rheumatoid arthritis; Dr. Alexander B. Gutman, director of the Department of Medicine, Mount Sinai Hospital, New York City, for his work on the treatment of gout; Dr. U. S. von Euler, professor of physiology, Karolinska Institutet, Stockholm, for his work on noradrenaline and its effects on body functions.

The Royal Institution of Naval Architects: Awards

THE Council of the Royal Institution of Naval Architects has announced the following awards: *Denny Scholarship in Marine Engineering* (1961), to Mr. David K. Brown (17), of Kelvinside Academy, Glasgow (£350 per annum for four years at the University of Glasgow); *Parsons Scholarship in Marine Engineering* (1961), to Mr. Alan B. Turner (21), of Messrs. Alfred Holt and Co., Ltd., Liverpool (£400 per annum for three years at the University of Durham, King's College, Newcastle upon Tyne).

The Meldola Medal for 1961

THE Meldola Medal is the gift of the Society of Maccabæans and is normally awarded annually. The next award will be made early in 1962 to the chemist who, being a British subject and under thirty years of age at December 31, 1961, shows the most promise as indicated by his or her published chemical work brought to the notice of the Council of the Royal Institute of Chemistry before December 31, 1961. No restrictions are placed upon the kind of chemical work or the place in which it is conducted. The merits of the work may be brought to the notice of the Council, either by persons who desire to recommend the candidate or by the candidate himself, by letter addressed to the President, Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

The Association of Clinical Biochemists

THE following officers were elected at the annual general meeting of the Association of Clinical Biochemists: *President*, Dr. A. L. Latner; *Chairman*, Mr. H. Varley; *Honorary Treasurer*, Dr. R. Gaddie; *Honorary Secretary*, Dr. J. H. Wilkinson, Westminster Medical School, Horseferry Road, London, S.W.1.

Announcements

DR. RALPH P. HUDSON has been appointed chief of the Heat Division at the National Bureau of Standards, U.S. Department of Commerce. He succeeds Dr. Charles M. Herzfeld, who was recently appointed associate director of the Bureau.

THERE will be open days at the Humber Laboratory at Hull of the Department of Scientific and Industrial Research on November 9 and 10. Exhibits will include some of the work of the parent laboratory, the Torry Research Station at Aberdeen. Both laboratories deal with the handling, processing and distribution of fish.

THE Welsh Soils Discussion Group will meet at the Department of Biochemistry and Soil Science, School of Agriculture, Bangor, on November 15. The subject for discussion is "Aspects of the Dynamic Organic Matter Cycle in Soils". Further information is available from Mr. J. A. Taylor, University College, Alexandra Road, Aberystwyth.

THE four hundred and tenth meeting of the Biochemical Society, for the presentation of original communications, will be held in the Department of Biochemistry, Manchester College of Science and Technology, on November 11. Further information can be obtained from Dr. W. J. Whelan, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1.

THE office of the Biochemical Society and the editorial office of *The Biochemical Journal* have now moved to 20 Park Crescent, Regent's Park, London, W.1. All communications connected with *The Biochemical Journal* should be sent to that address.

A SPECIALISTS' conference on culture collections will be held in Ottawa, Canada, during August 27–28, 1962. Topics for discussion will include fundamental and technical aspects of the preservation of culture of micro-organisms and other cells. Special emphasis will be placed on the maintenance of morphological, physiological and genetical characteristics. Attendance at the conference will be by invitation only. Persons interested in attending are invited to write to Dr. S. M. Martin, Division of Applied Biology, National Research Council, Ottawa, Canada, before December 1, 1961.

THE first Feldberg Foundation Lecture 1961 will be given by Prof. Herbert Hensel, director of the Physiological Institute of the University of Marburg, who will speak on "The Electro-physiology of Thermoreceptors" on November 2 in the Physiology Theatre, University College, London, Gower Street, W.C.1. The Foundation was recently instituted (see *Nature*, **191**, 1143; 1961) to encourage German–English scientific exchange for experimental medical research. Further information can be obtained from Dr. M. Godfrey, Medical Research Council, 20 Park Crescent, Regent's Park, London, W.1.

THE Czechoslovak Society of Arts and Sciences in America will hold its first national scientific congress in the Hotel Statler, Washington, D.C., during April 20–22, 1962. There will be a two-day symposium on the contributions of the Czechoslovak science and arts to world culture. The programme will include papers on science, medicine and technology; fine arts; literature and literary criticism; slavistics and linguistics; law and economics; history and political science; philosophy and sociology; and finally a special section will be devoted to Czechs and Slovaks abroad. Further information can be obtained from Dr. M. Rechcigl, jun., secretary, Programme Committee, 1703 Mark Lane, Rockville, Md.

XUM

DEVELOPMENTS IN ASTRONAUTICS

THOUGHTS on the seminar on "Astronautics", held in the University of Southampton during July 12-19, should be considered against the technology of the changing scene in air and space, an unsteady scene, its movements affected by engineering and scientific inventions. The trend is to curtail weapon programmes, and in place of production quantities there is more limited manufacture of precision 'hardware'; aircraft types are fewer and more highly specialized, benefiting from the development of rocket systems and instrumentation.

The five groups of twenty-three interrelated lectures provided a clear picture of the scientific and technological aspects of the subjects, the result of painstaking planning by those to whom science was an intellectual adventure.

The seminar was organized by the N.A.T.O. Advisory Group for Aeronautical Research and Development and the University of Southampton. Prof. E. J. Richards, head of the first Department of Aeronautics and Astronautics in Great Britain (University of Southampton), introduced Mr. E. T. Jones, deputy director of A.G.A.R.D., who welcomed the delegates, mentioning the fitness of the venue, the first of its kind, due to the enterprise of the professorial staff, particularly Dr. B. L. Clarkson.

Mr. M. J. Lighthill, director of the Royal Aircraft Establishment, gave the introductory lecture to about a hundred delegates from Europe and the United States. Brilliantly he set the scene, introducing the subjects to be discussed; dealing with aims and organization of pure, and applied, space science and technology. Space included the stars, planets, and everything known and predicted by the astronomers, to whom we are indebted for information, and with whom it is most desirable to co-operate.

In pure science we are concerned with perturbations of orbits, macrometeorites in orbit, the fluctuating environment of plasma, solar activity, solar streams, and radiation effects, Van Allen belts, and plasma propulsion.

In applied science we consider geodesy, navigation, transit satellites, observations, communications, geometric aspects of space, meteorology, information for synoptic meteorologists, the particular need for viewing clouds from above—thermal stability in satellites, solar cells, gyroscopes, and generally the application of discoveries useful for mankind. For the future, *Blue Streak* and *Black Knight* would help in the further observation of the ionosphere and re-entry research as well as contributing to the communication satellites plan, now being handled by the Royal Aircraft Establishment and the General Post Office, the Commonwealth and the European Space Launcher Organization; this scheme is promising and will engender greater integration, economical development, technical adventure and co-operation over a wide field, and would attract the best men. The co-operative scheme needs intensive planning for arranging the work, and a body to co-ordinate effort from design to the operating conditions. This includes using existing institutions, avoiding extravagances, and avoiding the separation of aerodynamics, astronautics and space technology.

Useful visits were made to the Royal Aircraft Establishment, the Radio Research Establishment, and the Royal Greenwich Observatory.

"Orbits in the Solar System" was the subject of a lecture by Dr. J. G. Porter, Royal Greenwich Observatory, and he was followed by Mr. D. G. King-Hele, Guided Weapons Department, Royal Aircraft Establishment, with a paper on "Earth-Satellite Orbits".

These papers dealt with orbits affected by perturbations. As an astronomer, Dr. Porter was concerned with all the members of the solar group and their movements in continuously variable ellipses, and the method of defining the instantaneous orbit in which a body is travelling at a given epoch. The figures in the published ephemerides of the Sun and planets are based on rectangular co-ordinates measured in astronomical units to seven decimal places; they are published so that observers may make a comparison with theory. Tables of planetary co-ordinates for the next twenty years are available, and for Mars for every four days up to 2,000.

Complementary to Dr. J. G. Porter's talk, Mr. D. G. King-Hele dealt in more detail with four main perturbations affecting the orbit of a satellite, caused by: (1) the departure of the Earth's gravitational field from spherical symmetry; (2) the influence of atmospheric drag; (3) the luni-solar gravitational attractions; and (4) solar radiation pressure. Near satellites, having a perigee height of less than 1,000 km., and apogee heights of less than 5,000 km., are affected particularly by 1 and 2. The third perturbation results from the asymmetry between the northern and southern hemispheres. If the apogee height of a satellite is greater than 20,000 km. (*Explorers* 5 and 10) the gravitational attractions of the Sun and Moon are important in addition to those already mentioned, and if the perigee height exceeds 1,000 km. the force due to solar radiation pressure is greater than atmospheric drag.

Prof. T. R. F. Nonweiler, Mechan professor of aeronautics and fluid mechanics, University of Glasgow, spoke on "Re-entry Orbits", discussing the two conditions in which re-entry will occur, either as the final result of the gradual contraction of the orbit by air resistance, or by deliberate perturbations (through the action of retro-rockets). The speed at the first stage of re-entry, say Mach 30, is changing to stage two at Mach 3 or 4, then to stage three at Mach 0·3–0·2, and finally the vertical drop. While drag can be reduced, it is not possible to reduce the rate of heating, and this problem at hypersonic speeds has two satisfactory solutions, namely, ablation, and variable drag by flared skirt or parachute. When retro-rockets are used to control the vehicle, the point of firing is important so that the vehicle hits the target. Whatever the shape of the vehicle the maximum deceleration is the same, but it occurs at a greater height if the design is blunt and is of high drag; thus in the lower part of the atmosphere a blunt satellite has a lower speed and endures less heating. A more streamline and faster-falling vehicle suffers intense heating in the denser part of the atmosphere where maximum deceleration

occurs. If the vehicle can be given some aerodynamic lift to modify the re-entry path, then the rate of heating and deceleration can be reduced.

Captain P. V. H. Weems, U.S. Naval Academy, rounded off the session on orbits by explaining his method of "Space Navigation". The basic concept is that the space observer's optical line of sight to the Earth's centre is in the observer's vertical. Range is restricted to cislunar navigation and the space-vehicle is assumed to have sufficient power to enable it to be directed and navigated. The Earth is used: (1) for fixing the position of the space observer's geographical position by observing visually the position of the Earth's centre relative to the stars; (2) for finding the distance from the Earth's surface stadimetrically by using the Earth's diameter as a base; (3) for guiding a space-vehicle by so changing its velocity as to cause the apparent path of the Earth's centre to follow a precomputed path. The space navigator can fix the relative position of the satellite by using a two-way telescope, which would provide, simultaneously, fixes on the Earth's centre and the zenithal stars, and then tables will give the position. The proposed *Space Almanac* has been started, by attempting to provide a space navigator for the *Echo* satellite with usable data, and it is intended for teachers and students one or two generations ahead of the astronaut.

Prof. E. Vassy, of the University of Paris, in discussing "Physical Properties of the Upper Atmosphere", paid due tribute to the astronomers, and outlined the results from the *Veronique* research rocket, the programme being similar to that of the *Skylark*, there being close collaboration with the Royal Aircraft Establishment in the development of the mass spectrograph for ion measurement.

Prof. B. Fraeijs de Veubeke, University of Liège, discussed the "Optimization of Space Vehicles and Launchers relative to their Missions", and showed that minimization of fuel consumption, initial weight, initial thrust for a given payload and given performance requirements lead to widely different missile configurations; and then there is the extensive analysis necessary to determine the minimization of cost of mission per unit payload.

Prof. L. Broglio, Department of Aeronautics, University of Rome, explained "A Method for Solving Non-linear Thermal Problems in the Re-entry of Space Vehicles".

In "Some Recent Advances in the Theory of Control", Dr. J. K. Lubbock, University of Cambridge, assessed the progress of control theory embracing non-linear systems, and dealt with the difficulties of analysis and optimization of feedback systems containing non-linear elements. He reviewed the Fokker–Planck equation for the Markov processes; he believes that Pontryagins' general theory is the most significant theoretical advance in recent years.

Dr. W. W. Lines, Guided Weapons Department, Royal Aircraft Establishment, outlined the "Problems of Guidance in Astronautics" and dealt with methods for the injection of Earth satellites into orbit, and the problem of orbital transfer.

Flight-Lieutenant J. C. Guignard, Institute of Aviation Medicine, discussed "Physiological Limitations of Man in Space". He said that from the physiological aspect, space begins at sea-level, and the basic problems extend to meet the conditions discussed in the seminar are being studied in simulation laboratories. The absence of normal diurnal and seasonal cycles during flights of weeks or months demands new knowledge.

Dr. E. V. D. Glazier, Royal Radar Establishment, discussed the radio problems for interplanetary space probe missions where the range may be many millions of miles, but at present there are limitations on probe transmitter power and life, and on the extent of costly ground facilities. Measurement of range is achieved by the secondary radar principle, and good tracking in range and angle can be obtained out to ten million miles with a slight extension of present techniques. For reciprocal and rapid transmission during a Venus mission, more powerful transmitters and aerials of the Jodrell Bank type are essential.

In a paper on "Hypersonic Aerodynamics", Mr. K. M. C. Bray, University of Southampton, said that perfect fluid hypersonic effects, real gas effects and high-altitude effects are three classes of problem that may occur simultaneously, but they must be studied separately in order to provide information for the design of the blunt-nosed ballistic vehicle or the slimmer boost glider type. The designer needs to know the heat-transfer rate at the stagnation point, perhaps in the presence of ablation, then data about drag and dynamic stability. Due to the Faraday effect, under hypersonic conditions radio signals to a vehicle suffer serious interference.

Complementary to Mr. K. M. C. Bray's paper was "Laboratory Facilities and Techniques for Rarefied Gasdynamics Research", by Mr. R. L. Chuan, director, Engineering Centre, University of Southern California, who stressed the need for more advanced designs of experimental apparatus to analyse the problems raised by Mr. Bray. Four types of facilities of various degrees of complexity and cost are needed: the wind tunnel, shock tubes, molecular beam, and the rotating arm. The study of hypersonic phenomena requires conditions in air of Mach number greater than 10, a Reynolds number less than 100, and a static temperature of about 300° K. Pressure, temperature, density, speed, gas composition are basic quantities to be measured, the pressure being of the order of 10^{-6} atm.; and this Mr. Chuan had achieved by designing a low-density hypersonic tunnel around a cryopump cooled by a helium refrigerator achieving 20° K.

Mr. D. J. Johns, College of Aeronautics, reviewed the design of the thin cylindrical structures typical of the liquid-fuelled launching vehicles, covering all the ground handling and flight conditions until impact on landing. They included pressurization, axial and lateral loads, thermal, dynamic and space environment effects. In general, the pressure-stabilized structure is lighter and simpler than the conventionally stiffened type. The inflatable structure has minimum structural density, maximum volume/weight ratio, and unmatched packageability for the launch phase. Prof. A. J. Murphy, principal of the College of Aeronautics, spoke about the nature of the environment in outer space, and its significance for "Materials for Astronautic Vehicles", and said that the requirements discussed by Mr. D. J. Johns could be met by the use of metals of higher melting point, especially beryllium, molybdenum, columbium and tungsten, and also non-metals such as carbon, ceramics, or metal–ceramic mixtures. Refractory metals withstand 1,370° C. at stressed conditions but need protection against oxidation. Heat-dissipating ablation surfaces of organic material are satisfactory.

XUM

Prof. E. J. Richards (Southampton) discussed "Some Problems of Noises in Ballistic Missiles", briefly sketching the effect of noise-levels on structures and instruments between 100 and 180 decibels; at 140–150 db. structures begin to crack, and at 170 db. cracking is immediate. To guard against trouble, there must be a detailed analysis of the environment of noise around a vehicle both during launching and at re-entry. The source of most efflux noise of a rocket is about eighteen diameters downstream, the unsteady conditions with fluctuating pressures arising from turbulent mixing of the gas in the jet, from the rough flow in the boundary layer, and from the movement of the missile in turbulent air; fluctuating shock waves buffet the missile. Noise may be real and radiated acoustically or as hydrodynamic pressure fluctuations exciting the structure and causing acoustical radiation inside it.

Mr. G. K. C. Pardoe, chief weapons research engineer, De Havilland Co., spoke on "Space Flight Projects based on *Blue Streak*". His practical outlook brought together the theoretical and design philosophies discussed in other papers. *Blue Streak* will have a fundamental part in the proposed research work on probes, satellite communication and meteorology, and this activity means that the European Space Launcher Organization should get off to a good start. Mr. Pardoe reviewed the *Eslo* three-stage launcher based on *Blue Streak*, with a second stage of French design. Various third stages have been proposed and could produce high or low thrust, depending on the kind of mission; *Black Knight* with Bristol Siddeley *Gamma* engine would give a versatile performance. Mr. C. R. Turner, of the De Havilland Co., spoke on "Communication Satellites", describing a world-wide coverage scheme based on *Blue Streak*.

Prof. P. C. Parkes, Control Engineering Department, University of Southampton, speaking in Russian, welcomed Prof. A. M. Bobrov, chief scientist, Astronomical Society, Academy of Sciences, U.S.S.R., who discussed the "Cosmological Neighbours of the Earth" from the astronomer's point of view, referring to the Sun, and the atmosphere of the Moon, Venus and Mars. Astrobiological laboratories are growing plants under simulated Martian conditions. A third belt of radiation has been located beyond the Van Allen belts.

Dr. R. R. Jamison, assistant chief engineer (ramjets), Bristol Siddeley Engines, Ltd., in discussing "Advanced Air-breathing Engines", said that the use of these engines for astronautical work was as propulsion units for space-vehicles during the boost phase of space missions. Studies based on development work show that the ramjet has a better fuel specific impulse than a typical rocket engine and so should be a better first-stage booster, faster and lighter. It must operate up to speeds which are a significant proportion of orbital velocity, and research indicates that speeds much higher than the present operable Mach 6 can be achieved.

Mr. S. L. Bragg, chief scientist, Rolls Royce, Ltd., discussed "Operation and Control of Liquid Propellant Rocket Engines", and explained the difficult handling problems of starting, keeping the engine running, and stopping. The *Blue Streak* engine is the *RZ*-2, using liquid oxygen and kerosene. Accurate control of the moment at which the engine is shut down is essential to enable the accelerating vehicle to follow the flight plan. After the stopping signal, there will be a brief time while the engine runs down, and on a multi-stage rocket it is desirable that the thrust should cease before the stages separate to avoid the possibility of subsequent collisions.

Mr. P. A. E. Stewart, rocket and satellite systems engineer, Hawker Siddeley Aviation, Ltd., in dealing with "Advanced Propulsion Systems for Space", reviewed the three main classes: (*a*) high acceleration, covering free-radical rocket propulsion; open-cycle thermonuclear propulsion, and ionospheric recombination ramjets; (*b*) for medium acceleration there is solar energy; and (*c*) for low acceleration the closed-cycle nuclear system and variations of the electrodynamic types, electro-thermal or arc-jet, electrostatic or ion engine, the magnetohydrodynamic type, and the photon system.

Group (*c*) attracted most interest; progressive laboratory work has been achieved in electrical propulsion and the arc-jet is well advanced. Electric propulsion provides jet velocities 5–10 times those of nuclear engines, but as the thrusts are limited to pounds and fractions of a pound, they are promising only in space flight; but the problems are chemical and mechanical. Then there is the question of space-flight test-beds.

A. Cadman Clinton

THE COLONIAL DEVELOPMENT CORPORATION

Annual Report, 1960

THE annual report and statement of accounts of the Colonial Development Corporation for the year ended December 31, 1960, the first to appear over Lord Howick's signature, who, as chairman, succeeded Sir Nutcombe Hume on December 1, 1960, follows the pattern of recent reports*. Ninety-one continuing projects are reported as compared with 88 in the previous year, including 10 new projects totalling £11,675,000. Seven projects have been removed from the list and total commitments aggregated £109,448,000 compared with £96,145,000 in 1959. Of this aggregate, £14·6 million was in the Caribbean; £19·4 million (including £12·8 million for the Federation of Malaya) in the Far East; £22·7 million in East Africa; £22·0 million in Central Africa; £16·0 million in the High Commission Territories; and £14·7 million (including £205,000 in Ghana and £8·9 million in the Federation of Nigeria). Functionally 52·5 per cent of the committed capital was for basic development, 38·3 per cent for primary production and processing, and 9·2 per cent for commerce and industry.

The report is at pains to emphasize the nature of the Corporation's task and some of its achievements in Africa. It is believed that the Corporation can make an important contribution to the economic life of the emerging peoples for several reasons. Its regional organizations have now been established for nearly ten years and its employees are able to

* Colonial Development Corporation. Annual Report and Statement of Accounts for the year ended 31st December, 1960. Pp. vi+82. (London: H.M.S.O., 1961.) 5*s.* net.

relate business needs to the conditions of the region. They have come to know well some leading people in the political and commercial life of their region, and are accustomed to working with them. The Corporation is able either to take a shareholding or make a loan according to whether the principal need of a country is, at one time, for an equity type investment or, at another time, for a loan, and those in charge of the Corporation have become accustomed to working with many partners and have learned, for example, the importance of the pilot scheme stage in development projects.

The achievements of the Corporation in Africa are illustrated by reference to the Mhlume Sugar Mill in Swaziland, built in partnership with Sir J. L. Hulett and Sons, on a Corporation irrigation project, which will have produced by the end of 1961 more than 40,000 tons of sugar; the new pulp mill at Usutu Forests in the same territory, with an annual output of 100,000 tons ; the Lobatsi abattoir in the Bechuanaland Protectorate; the launching of the Kilombero Sugar Co., Ltd., in Tanganyika, with the International Finance Corporation, Dutch interests and the Standard Bank of South Africa; and the agreements concluded in Nyasaland to provide up to £1,300,000 for the Mudi River Water Board to relieve the water shortage in Blantyre and Limbe. The value of the Corporation's activities has equally been clear in countries which have become independent since the Corporation was established.

As regards future possibilities, the report stresses particularly that, while remaining fully commercial, the Corporation could and should give the help which smallholders need in loans in processing and marketing their produce and in establishing a few larger farms for demonstration and for experiments with new agricultural methods, because development of smallholder farming can contribute powerfully to the stability of a country. In small or sparsely inhabited territory the Corporation can often do much and is frequently irreplaceable, as is shown by the hydroelectric scheme in Dominica and in St. Vincent established by the Corporation, which provided the background for production of bananas, cocoa and copra. Great importance is attached to the training of men in the territories in which the Corporation operates to undertake posts of responsibility both in local projects and the Corporation's organization as a whole. The Corporation believes that the best training is that received on the job provided a real effort is made to explain and demonstrate by those appointed to supervise, but in view of the absence of minimum educational background in many territories the Corporation decided last year to offer, on a very limited basis, scholarships to promising students as a contribution towards solving the educational difficulty.

When the Corporation's report was discussed in the House of Lords on June 27, the Earl of Perth emphasized that in the main this was a matter which should be handled through the new Technical Aid Department, and that the Corporation should limit itself to giving training and occasional scholarships to particular people who were to work on a particular project. Generally, warm approval of the work of the Corporation was expressed in the debate, criticism centring rather on the Government's financial policy to the Corporation and the delay in giving effect to the recommendations of the Sinclair Report. The Earl of Perth, however, mentioned that the Government had followed closely the essential recommendations regarding stock representing loans advanced for 'finance house' business and for stock representing abandoned schemes and fructification interest, but laid down that repayment for this stock should depend on the Corporation, after its various obligations, having a profit of more than £250,000. The only variation was in respect of the recommendation for '*B*' Stock, which involved a fluctuating subsidy and thus no Parliamentary control, and here the Government insisted that the agreed rate must be paid; but as these investments would be ready for equity investment the Government would continue with the fructification period for seven years. The suggestion for a reserve or allowance for exploration was met by allowing the Corporation to use the first £250,000 of its true profits for whatever purpose it saw fit. Lord Perth also said that it was not expected that the Corporation, which was not at the moment in need of funds, would require increasing funds indefinitely: it might be expected from time to time to sell off successful ventures. He thought the Corporation would still be able to play a limited part in the newly independent countries, but that it was right that the Corporation should concentrate on the remaining Colonial territories.

Research Schemes and Loans, 1960

The schemes made under the Colonial Development and Welfare Acts in the period April 1, 1960–March 31, 1961, which are detailed in the latest returns*, bring the total commitments from April 1, 1946, under the Acts to £275,318,622, of which £20,798,424 is for research schemes. The totals for the year in question are £35,291,739 for development and welfare and £2,186,869 for research schemes. Of the latter, £1,085,365 is for agriculture, £400,624 for medicine, £223,784 for tsetse research and trypanosomiasis, £150,786 for social science, £108,360 for fisheries, £56,401 for pesticides, £25,727 for locust control, and £6,803 for economic research.

Of the research schemes, £446,149 is towards the cost of research organizations controlled by the East African Agriculture and Fisheries Research Council, but there are other considerable contributions to the cost of work sponsored by the Medical Research Council, the Agricultural Research Council or regional organizations; for example, £30,000 for the Medical Research Council's laboratories at Fajara, Gambia, and £11,750 for entomological research there; £133,516 for continuation of services, other than the Leprosy Research Centre controlled by the East African Council for Medical Research, 1960–63; £27,432 to the West African Council for Medical Research and its associated units; £27,103 to the West African Building Research Institute; £22,412 to the West African Institute for Trypanosomiasis Research, 1960–61; £43,320 for the Regional Virus Research Laboratory, Trinidad; £148,000 for the Regional Research Centre, Faculty of Agriculture, University College of the West Indies; and £8,000 to the Agricultural Research Council Unit of Experimental Agronomy and Weed Research Organization, 1961–62.

Other major grants which may be noted are: £42,827 for immunological work on trypanosomiasis and preparation of precipitin sera at the Lister

* Colonial Development and Welfare Act: Return of Schemes made and of Loans approved under the Colonial Development and Welfare Act in the period from 1st April, 1960, to 31st March, 1961. (House of Commons Paper 240). Pp. 58. (London: H.M.S.O., 1961.) 3*s*. 6*d*. net.

Institute of Preventive Medicine; £39,898 for tsetse fly breeding at the University of Bristol; £34,303 for continuation of the preparation of the Flora of Tropical East Africa; £62,933 for continuation and expansion of agricultural research and land-use planning in Tanganyika; £62,645 for the Agricultural Research and Experimental Station, Nyasaland; £56,550 for establishment of a Citrus Research Unit in the British Caribbean; £79,549 for continuation of the Institute of Social and Economic Research in the West Indies; £48,812 for the Tropical Metabolism Research Unit in Jamaica; and £20,934 for soil surveys in British Guiana.

Development and welfare schemes of scientific interest include various contributions to Royal College, Nairobi, and the University College of East Africa, Makerere, as well as to the University College of the West Indies, and £450,000 for the construction of a College of Arts, Science and Technology, Malta, which received grants totalling more than £5 million during the year; £421,907 for intensification of African agriculture under the Swynnerton plan in Kenya, and a further £102,804 for the Veterinary Department; £518,300 for forestry development in Tanganyika, and £148,445 for continuation and expansion of agricultural research and land-use planning; £246,000 for development of broadcasting in Uganda; and £429,132 for soil conservation and land-use planning in Basutoland, 1960–64; £18,877 was contributed for the activities of the Commission for Technical Co-operation in Africa South of the Sahara and the Scientific Council for Africa South of the Sahara, and there were numerous contributions for education at secondary and primary levels.

THE NORFOLK BROADS

ON August 1, the Minister of Housing and Local Government announced in the House of Commons that he had accepted the advice of the National Parks Commission that the unique character and complex problems of the Norfolk Broads make them unsuitable for designation as a National Park. It was therefore appropriate and timely that the British Association should discuss some of these problems during its Norwich meeting. Sections E (Geography) and K (Botany) held a joint symposium on September 1 at which the main contributions were made by Dr. Joyce Lambert of the Botany Department, University of Southampton, Mr. C. T. Smith of the Geography Department of the University of Cambridge, and Mr. Charles Green, an archæological consultant to the Ministry of Works.

The factors which render the Broads unsuitable as a National Park stem largely from the rapid changes which are taking place at the present time and which are all recent in effect, if not in origin. The silting up of the Broads, with the consequent overgrowth by vegetation had scarcely begun to reduce the area of open water when the Tithe Commission mapped the area in the mid-nineteenth century. The degeneration of mowing marsh into scrub and carr follows changes in agricultural practice in the past half-century. These physiographical and ecological developments clearly have a profound significance in terms of the ever-increasing use of the Broads as a holiday area, a use which introduces its own problems, both ecological and economic. While these changes have only recently forced themselves on the attention of students of the broadland scene, they are bound up with the basic problems concerning the age and origin of the Broads, which formed major themes in the Norwich discussion and which involve rather longer periods of time.

Most nineteenth-century writers from Robbards and Taylor in the 1820's to Gregory in 1892, sought to interpret the Broads as residual natural lakes resulting from the silting up of a 'great estuary', the three arms of which occupied the valleys of the Rivers Waveney, Yare and Bure. Many of these writers assumed that the 'great estuary' persisted well into historical times. A few workers sought to invoke glacial agencies. In 1952, J. N. Jennings published a monograph on *The Origin of the Broads* (No. 2 in the Royal Geographical Society's Research Series). This was the first weighty piece of original research to appear for almost half a century and represented the results of several years labours, mainly in the valleys of the Bure and its tributary, the Ant. Jennings suggested that the Broads can be explained in terms of a geologically very recent marine transgression during which a thick wedge of estuarine clay was laid down over the early valley peats of the Norfolk river valleys. He suggested that the Broads themselves were initiated as discontinuous lakes, lying in natural hollows in the peat, filled with water ponded back between the flanges of the clay and the upland valley margins (by-passed broads) or in tributary valleys the mouths of which were blocked by the clay (side-valley broads). Jennings examined the possibility, mooted on several occasions to explain particular broads, that all the Broads were man-made. For a variety of reasons he came to the conclusion that "a general theory based on peat cutting appears to be wholly unacceptable".

Almost before his monograph was in print, Jennings had modified his views in the light of new work. Dr. Lambert, with whom he had collaborated closely in work on the valleys of the Bure and the Ant, had obtained convincing evidence that the Broads of the Yare valley are artificial. She employed the same boring methods and the same pollen analysis technique, to determine the stratigraphy of the clays, peats and muds, as Jennings had used. A reconsideration of the evidence from the Bure and the Ant valleys suggested that deep extraction of peat in historical times was responsible for all the by-passed broads, though, for lack of evidence, the side-valley broads were not as yet included in the general theory. This recognition of the fact that most, if not all, of the Broads are man-made did not, of course, detract from Jennings's interpretation of the natural evolution of the river valleys in which the Broads are located.

In 1960, Jennings and Lambert published a re-appraisal of the whole problem of the evolution of the Broads. They found it "difficult, in view of the stratigraphical evidence to conceive a substantial alternative" to the theory that all the Broads are deep peat diggings which have been afterwards flooded. Breydon Water is excluded from this explanation since it is clearly not a broad in the accepted sense and is merely the surviving part of a

formerly more extensive Bure–Yare–Waveney estuary.

Evidence from the field of historical geography assembled by Mr. C. T. Smith and the results of archæological work, interpreted by Mr. Charles Green and Mr. J. N. Hutchinson, supported the new hypothesis. The combined evidence was published under the title *The Making of the Broads* (No. 3 in the Royal Geographical Society's Research Series).

At the Norwich symposium, Dr. Lambert reviewed the physiographical and botanical evidence on which their findings are based. Boring has revealed that the basins in which the Broads lie have uniformly steep sides and virtually level floors with resulting rectangular profiles. Islands and peninsulas with similar vertical sides have exactly the same stratigraphy as the surrounding fenland. They are interpreted as balks left in the extraction of peat to a depth of 10–12 ft. In old surveys, including tithe and enclosure maps and in modern air photographs these islands and peninsulas can be correlated with boundaries between holdings.

Two of the more important difficulties in accepting human agencies to account for the existence of the Broads relate to the enormous amount of peat involved and the need to postulate changes in water-level, which would permit the excavation of peat to depths well below those of present-day workings, and eventually lead to the flooding of the pits. Mr. C. T. Smith described the search for evidence which would resolve these difficulties. Nothing in the published records of broadland suggested an 'artificial' origin. The large-scale changes caused by the extraction of an estimated nine hundred million cubic feet of peat and the flooding of the workings appear to have made no impact on local tradition and folk-lore. However, a careful and detailed study of documents and maps supplied strong circumstantial evidence in support of the deductions based on the stratigraphy. References to turbary and the sale and consumption of turves show that large-scale peat digging was well under way by the twelfth century. Much of the consumption must have gone unrecorded but examination of terriers, deeds, account rolls and registers, such as those of Norwich Cathedral Priory and St. Benet's Abbey, give an indication of the scale of operations. The purchase of 400,000 turves by Norwich Cathedral Priory in 1316 is typical. These same records also show that by the beginning of the fourteenth century the flooding of the turf pits had begun. While extraction continued well into the fifteenth century it was increasingly difficult and expensive, and involved first the dredging of peat with simple scoops and then the making of turves. Soon other fuels replaced turves in the records and references to turbaries were replaced by references to open water and fisheries.

The archæological evidence put forward by Mr. Charles Green showed that emergence from the Romano–British submergence reached a peak in the thirteenth century. The excavations on the site of the power-station at South Denes, Great Yarmouth, indicate a relative fall in sea-level of the order of 13 ft. This means that deep extraction of peat from the Norfolk valley floors was possible in 'dry' pits. Similar evidence revealed a reversal of the downward movement of sea-level, initiated toward the end of the thirteenth century, which clearly accounts for the slow but inexorable flooding of the peat-diggings and the formation of what we now call the Norfolk Broads.

As a piece of scientific detection, the story unfolded to the Norwich symposium was a masterpiece. It is, however, not only of historical or academic interest. The state of dynamic change, which Dr. Lambert showed to underlie all the present-day problems of the Broads, both scientific and practical, stems directly from these surprising beginnings. The vegetation of broadland does not, as was formerly thought, result from the overgrowing of large continuous sheets of open water. There is a fundamental distinction between the vegetation which has grown up on the solid fen, much modified by a variety of human interference, and that which is encroaching on the basin of the Broads. The filling of the man-made lakes with freshwater mud, which has made this encroachment possible, only reached a critical stage about the beginning of the nineteenth century. It has, however, already resulted in a rapid reduction of the open water available to the holiday maker. Dr. Lambert is continuing her ecological work in the Broads, but the number of problems which still remain to be tackled, some of which she touched on, is very large. The need for more research was the theme of the discussion which followed the reading of papers. Any new work which may be instituted will owe much to Dr. Lambert and Mr. Jennings and their colleagues.

C. KIDSON

THE SOIL AS AN ENVIRONMENT FOR ANIMAL LIFE

A SYMPOSIUM on "The Soil as an Environment for Animal Life" was held on September 1 by Section D (Zoology) at the Norwich meeting of the British Association for the Advancement of Science.

Sir John Russell pointed out that soil contains four groups of components: a mineral framework, organic matter, mineral matter from the bodies of organisms and soil solution. The relative proportions of organic and inorganic substances largely determine the suitability of the soil as a habitat: arable soils, with the lowest organic content, contain fewest organisms, while grasslands are usually richer.

The organic matter is derived from plants, their remains being reduced by organisms in the soil to their original simple origins: residues remaining form the humus. This is black, has colloidal properties, and in the climate of Great Britain contains about ten times as much carbon as nitrogen. If non-nitrogenous substances are added to the soil, atmospheric nitrogen is fixed, while if nitrogenous substances are added the humus splits off nitrogen as ammonia or nitrogen gas.

The mineral framework of the soil contains particles ranging from 2 mm. to 0·02 mm. in equivalent diameter: these are the sand and silt. Clay particles less than 2μ in equivalent diameter differ from the larger particles; they carry a negative electric charge, and can attract positively charged ions such as calcium and hold them against leaching. Clay is colloidal, with a great power of absorbing water: it expands on wetting and shrinks on drying, producing large cracks in the soil.

XUM

Calcium compounds, especially calcium carbonate, are as important as clay and organic matter in their biotic effects. While calcium carbonate is present the soil remains neutral, but as it dissolves in rain-water containing carbon dioxide and is leached out the soil becomes acid. The flora and fauna then become more restricted, though some organisms, such as *Plasmodiophora* and the amœbæ, do well. At a certain stage earthworms disappear: plant residues then remain on the surface and decompose there, forming 'mor'.

Organic and mineral soil components mingle to form crumbs, the optimum crumb size for plant growth ranging from 5 to 1 mm. Good crumbs are stable even under rainfall, while bad ones break down in wet conditions, forming a slurry. Mineral particles and crumbs have pore spaces between them, the total pore space being increased by crumb formation.

For organisms to survive in the soil, five conditions are necessary: living space, air, water, suitable temperature and freedom from injurious factors. The pore spaces provide the living room, and are also the water reservoirs. The chief cause of water loss is suction by plant roots: evaporation causes less than might be supposed. The surface layer dries quickly and protects the soil beneath, and there is no evidence of capillary movement of water from wetter to drier parts except just above the water table.

Diffusion of air into the pore spaces is very rapid, and the composition of air 6 in. down on arable land differs little from that of the atmosphere, except that the amount of carbon dioxide is greater, reaching 0·1 per cent on bare soil and 0·2 per cent under crops. In grassland soil the carbon dioxide content is greater: 3 per cent has been recorded. This high concentration does not appear to affect the soil population seriously.

Soil temperatures reflect those of the air, but fluctuations are heavily damped. Variations are greater on arable land than on grassland.

Harmful factors are numerous and may be drastic. These include acids, products of anaerobic decomposition such as hydrogen sulphide, the presence of parasites and predators, etc. Soil bacteria act as scavengers, removing harmful substances very effectively.

The question of food for the soil fauna has been studied most fully by the foresters. Leaves and twigs form litter on the soil surface: in it a mixed population develops and makes the primary attack on the food material it contains. Then follows the secondary decomposition, in which organisms nurtured in the first stage are eaten by predators that either capture their prey or poison it and feed on its decomposing body; also the excretions of primary decomposers are eaten by coprophagous animals. The final residue is humus; this persists in the soil, but is slowly decomposed, mainly by bacteria, the final end-products being carbon dioxide, ammonia, water and a few mineral salts. These will be starting material for a new generation of plants.

Dr. F. Raw discussed "The Interdependence of the Soil and its Fauna". The soil can be considered an environment created by animal activity from weathered mineral substrate and plant remains. The material from which the soil developed, the climate and the vegetation influence the animal communities developing in the soil, and these in turn influence the soil profile. Agricultural practices that affect the fauna may produce great changes in the biological and physical properties of the soil.

The greatest diversity and abundance of soil animals are found in virgin habitats such as permanent grassland or woodland. The arthropods in a permanent grass field in Cambridgeshire included 17 orders or classes, including uncommon groups such as the Pauropoda and Protura. Smaller arthropods were especially numerous. About 70 per cent of the Arthropoda occurred in the top 6 in., though some groups were more numerous at lower levels. In general, the smaller forms live deeper in the soil.

In arable soil the arthropod population is greatly reduced. In the Barnfield mangold plots at Rothamsted, the plots receiving mineral fertilizers yielded about 15,000 individuals per square yard, while in the dunged plots the number of individuals was almost twice as great, and the population was more diverse. The same was true of the earthworms, though the dominant *Lumbricus terrestris* was less affected by dunging than other species.

Earthworms are the principal agents that bury plant remains, mix them with the soil and so accelerate decomposition and the release of plant nutrients. In undisturbed habitats their activity can have a profound effect on the soil profile, and their presence or absence can result in the development of two fundamentally different soil types, 'mull' and 'mor'. This was illustrated by measuring the amount of leaf litter buried by worms in several apple orchards near Wisbech, by placing a known number of weighed leaves on the ground beneath wire cages at leaf-fall and recording the number and weight of leaves remaining in the spring. In grass orchards the number and weight of leaves buried was closely correlated with the estimated fresh weight of *Lumbricus terrestris*. Some orchards had about 20 cwt. of *L. terrestris* per acre, which buried 8–9 cwt. dry weight of leaves—enough to bury all the leaf-fall. Cultivation did not appear to affect the abundance of *L. terrestris* greatly, nor did regular applications of farmyard manure or compost.

In a grass orchard with a long history of heavy spraying with copper fungicides *L. terrestris* was not found, although it was abundant in surrounding unsprayed soil. In this orchard no leaves were buried; they decomposed slowly on the surface, and a mat of partially decomposed leaves, about 2 in. thick, had accumulated, so that the soil profile had typical 'mor' characteristics.

"The Soil Fauna and its Contribution to Fertility" was the subject of Dr. A. Macfadyen's paper. The soil contains enormous numbers of individual animals, and their action appears to be three-fold. The larger species tunnel through the soil and carry litter downwards and mineral matter upwards: at the same time they aerate and drain the soil and, by crumb formation and in other ways, render it a good medium for the growth of higher plants and micro-organisms.

The second function performed by the soil animals is a direct chemical one due to their feeding activities. They assist in the breakdown of litter in the soil, though here they make a poor showing compared with the micro-organisms.

The third property of the soil fauna has only recently come under investigation. It appears that, quite apart from their own direct metabolic effects on litter breakdown, soil animals act as catalysts of the metabolism of the bacteria, fungi and other micro-organisms. This catalytic action appears to operate through the cropping of senescent colonies, the re-distribution of slow-moving food materials, the

local removal of antibiotic substances, the re-concentration of nutrients and the spreading of spores to places where they can flourish. The biological properties of the microbes are such that high-energy food materials become inaccessible and energy becomes dammed up. The animals are the 'dam-busters' that disperse the accumulations of energy and restore the flow—and with it the nutrients needed by the higher plants.

It is not known yet how important these catalytic actions are, nor which of them are the most significant. Clearly, over the whole range of soil types, from forest and pasture to moorland and degraded arable land, the details of the picture are going to vary enormously; but the few experiments reported indicate that the animals' 'catalytic' role greatly exceeds their metabolic role as decomposers.

Current experiments are aimed at building up a good knowledge of the metabolic rates of isolated organisms under conditions as close to those in Nature as possible. At the same time systems are being developed containing micro-organisms and essential soil constituents of varying degrees of complexity but with predictable metabolic behaviour. It is hoped to go on from there to introduce different kinds of animals into the cultures and observe their effects on total metabolism, and to analyse the biochemical basis of these effects. In this way it is planned to bridge the gap between field conditions and laboratory experiments.

Dr. C. L. Duddington discussed "Predacious Fungi and Soil Eelworms". Predacious fungi are micro-fungi that attack small animals, including protozoa, rotifiers and eelworms. They are abundant in most fertile soils. We can distinguish two kinds of predacious activity among the fungi that attack eelworms. Some capture eelworms alive, while others are internally parasitic, attacking the host through the agency of sticky spores or by other means. At least two species of *Nematoctonus* combine both methods. Most of the work on predacious fungi attacking eelworms has been concentrated on the trap-forming species.

In the common *Arthrobotrys oligospora* and others the eelworm traps consist of systems of sticky networks, formed from curled, anastomosing branches of the mycelium. Eelworms that happen to touch the networks stick to them like flies on a fly-paper. Other species, like *Dactylella cionopaga*, have short, sticky lateral branches, while in *D. ellipsospora* and others small sticky knobs are attached to the mycelium by short stalks.

Some predacious fungi have mechanical traps. In *Dactylaria candida* rings, composed of three curved cells, are attached to the mycelium by short stalks; eelworms accidentally pushing their heads into the rings become wedged. In some species, such as *Dactylella doedycoides*, the cells of the rings are sensitive to touch; if an eelworm passes its body into the ring the three cells swell suddenly inwards, holding the eelworm by constriction.

Whatever trapping mechanism is used, the end result is that the eelworm is killed, and its body is invaded by hyphæ that consume its contents.

A survey of 49 British arable soils produced 82 records of predacious fungi, belonging to 20 different species. *Arthrobotrys oligospora* was the commonest: both eelworm-trapping and internally parasitic species were represented. There is direct evidence that predacious fungi prey on eelworms in the soil under natural conditions, for mycelium with traps and captives has been found on slides immersed in soil both in field plots and in the laboratory. There is also convincing evidence that their predacious activity is enhanced by the presence of decomposable organic matter in the soil. There is reason to believe that the organic matter affects the activity of the fungi directly and not, as has formerly been supposed, through its stimulating effect on the population of free-living eelworms in the soil.

The importance of predacious fungi in limiting the soil nematode population under natural conditions has yet to be fully investigated, but experiments on the use of predacious fungi for the biological control of plant pathogenic eelworms suggest that their effect is not negligible. Soil treatment designed to enhance the activity of predacious fungi was successful in controlling pineapple root-knot eelworm in Hawaii, and in Britain the number of larvæ of cereal root eelworm invading the roots of oat seedlings has been significantly reduced by soil inoculation with *Dactylella thaumasia* combined with the addition of organic matter to stimulate the fungus.

C. L. DUDDINGTON

DOMESTIC FOOD CONSUMPTION AND EXPENDITURE, 1959

THE survey for 1959 of the National Food Survey Committee* completes twenty years of continuous recording of domestic food consumption in Britain. Two reports cover the years 1940–49 and since 1950 a report has been published each year. These twenty years have seen a complete change from severe restriction in food supplies, followed by a period of gradual increase, to the point recently reached at which the housewife with money to spare looks for greater variety and shows a preference for what may be called 'convenience foods', processed and packed to save time and trouble.

A gap necessarily intervenes between the year of survey and the publication of these annual reports. As a result, they often clarify and extend facts already known in a general way. For example, the present report throws light on the increase in expenditure on food known to be taking place during the period under review. The average weekly expenditure a head increased from 28*s*. 5*d*. in 1958 to 29*s*. 3*d*. in 1959. This was partly due to an increase in the price of foods such as butter, meat, cheese and fruit, but there was also a shift in demand from staple foods such as bread, flour, potatoes and fresh fish to fresh fruit, fresh green vegetables and a wide variety of processed foods. Expenditure on processed foods prepared ready for the table, which include canned and frozen foods, cakes, pastries and biscuits, has risen from 4*s*. 3*d*. a head a week in 1955 to 5*s*. 5*d*. in 1959. Doubtless, as the range of such foods increases and advertising becomes more clamorous,

*Ministry of Agriculture, Fisheries and Food. Domestic Food Consumption and Expenditure 1959: Annual Report of the National Food Survey Committee. Pp. vi+142. (London: H.M.S.O. 1961.) 8*s*. 6*d*. net.

the expenditure on them will continue to rise, though a certain lack of variety in the taste of foods prepared by mass-cooking methods may eventually drive some housewives back to the kitchen stove. In the section dealing with the effect of the housewife's age on the household diet, it is shown that young housewives devote up to 19–24 per cent of their total food expenditure to these 'convenience' foods, while older housewives devote considerably less. The fact that 'single-earner' families with two children spend more on them than childless couples shows how useful they can be to the busy mother. Their cost in terms of food is increased by packaging and advertising, but this is offset by saving in time and less wastage. The expenditure on cooked and canned meats rose 5 per cent from the previous year, and on prepared fish the rise was 14 per cent. The most striking change in food habits in Britain, apart from the growing preference for processed foods, is the increase in consumption of poultry. Total supplies of poultry and rabbits are 31 per cent higher than pre-war, although there has been a considerable decrease in supplies of rabbit.

Comparison of the nutritive value of the diets of the different social classes with the recommendations of the British Medical Association showed that intakes of all nutrients, except protein, considerably exceeded the figures of the British Medical Association. Total protein intakes were 'borderline' in the lower income groups and the national average 99 per cent of the recommendation of the Association. As income rises, the calorie value of diets and their content of all the nutrients also rises. The diet of the most prosperous classes is 'richer' in these respects than that of all other classes. This perhaps suggests that wealthy people eat too much, but on the other hand it might be taken to imply that the recommended allowances are set too low. Such questions could be clarified only by correlating national food consumption data with data on the health of different sections of the population—an extremely difficult task.

Study of the relation between food consumption and family composition and size gave a less favourable picture. In households containing four or more children the diets were inadequate in certain respects. Intake of calories was 94 per cent of the recommended level, and that of protein, calcium and riboflavin 82, 81 and 95 per cent respectively. These deficiencies may not seem great, but it must be remembered that these families contain a high proportion of growing children whose health and development depend largely on proper feeding. At the other end of the life-scale an improvement is recorded. In earlier reports it was shown that the diets of old age pensioners were unsatisfactory ; it is good to note that their diets included in this survey were adequate for all nutrients.

Interesting facts are given about geographical differences in food consumption. These give rise to many fascinating speculations on feeding and cooking habits in different parts of Britain, but it would be foolhardy to hazard guesses on the merits and efficiency of housewives according to locality.

Joyce Doughty

NITROGEN IN SHEEP SALIVA

In a series of articles, M. Somers, of the Institute of Agriculture, University of Western Australia, describes the factors influencing the secretion of nitrogen in sheep saliva (*Austral. J. Exp. Biol. and Med. Sci.*, **39**, Part 2; April 1961).

Measurements were first made on the distribution of nitrogen in the mixed and parotid salivary secretion of sheep and on the amount of nitrogen secreted daily in the parotid saliva. The distribution of nitrogen in the mixed saliva followed the same pattern as that in the parotid secretions obtained when the glands were in a quiescent state. The concentration of nitrogen in both fluids depended on the rate of secretion. Although the concentration of total nitrogen in the mixed and parotid saliva increased during short-term stimulation, the pattern of nitrogen distribution was not affected. During prolonged stimulation of the parotid glands, the nitrogen composition of the saliva changed.

Quantitatively, urea was the most important nitrogen compound in both the mixed and parotid saliva, accounting for 60–70 per cent of total nitrogen. The average volume of saliva secreted daily by one parotid gland was 2,380 ± 43·8 ml. and the average total nitrogen content was 295·5 ± 5·77 mgm. when the nitrogen intake was 14·8 gm./day.

Somers later examined the influence of nitrogen intake on blood urea nitrogen and on the total nitrogen and urea nitrogen in the parotid saliva of sheep. Urea was added to the rumen of three merino sheep at the rates of 5, 10 and 20 gm. daily to increase the overall nitrogen intakes from 10·7 gm. in the basal ration to 13·0, 15·4 and 20·0 gm. a day. A marked increase in the total amount of nitrogen secreted in the parotid saliva was found to occur as the nitrogen intake of the sheep increased. A similar high correlation was found between the level of nitrogen intake and the amount of urea nitrogen secreted in the saliva daily.

Between the highest and the lowest levels of nitrogen intake the magnitude of the increases were 0·26 gm. of total nitrogen and 0·18 gm. of urea nitrogen/gland/day. The concentration of urea nitrogen in the blood increased with the level of nitrogen intake of the sheep, but fluctuations in the level of blood urea could not be accounted for by nitrogen intake alone. A significant correlation was found between the concentrations of urea nitrogen in the blood and in the saliva. This offered a partial explanation of the higher concentrations of salivary urea resulting from the increased nitrogen intakes.

Further investigations were made of the factors affecting the nitrogen fractions in the parotid saliva of sheep with special reference to the influence of production of ammonia in the rumen and fluctuations in level of blood urea. Quantitative measurements were made on the volume of saliva secreted hourly by the parotid glands of Merino sheep over a period of 24 hr. on three separate days. At the same time fluctuations in the concentration of urea and total nitrogen in the saliva were studied in relation to the production of ammonia in the rumen and changes in the level of blood urea which occurred throughout the day.

Levels of both rumen ammonia and blood urea followed a relatively simple cyclical pattern over

each 24-hr. period. However, two distinct phases were apparent in the salivary nitrogen cycle. The first, a short phase, followed the changes in stimulation associated with eating. The second phase was prolonged and more complex. It was influenced by changes in the level of blood urea, but additional factors also operated in the second phase.

Finally, Somers examined the influence of injected urea on the quantitative recovery of urea in the parotid saliva and the urinary excretions of sheep. Differential responses occurred in the amount of urea nitrogen and total nitrogen secreted in the parotid saliva, according to the nitrogen status of the sheep, when 1·4 gm. of urea nitrogen was injected into the blood daily. Of this amount of nitrogen, 0·1 gm. could be recovered in the secretion from one parotid gland daily when the sheep were in positive nitrogen balance, and 0·3 gm. when the sheep were in negative nitrogen balance. The urinary excretion accounted for 1·0 gm. of the 1·4 gm. of urea nitrogen injected daily when the sheep were in positive nitrogen balance, but only 0·3 gm./day at the lower nitrogen status. The implications of these findings have been considered in relation to a urea conservation mechanism in sheep.

CEREBRAL INFARCTION

IN 1959, in England and Wales, cerebrovascular disease was the certified cause of 75,150 deaths (14 per cent of the total mortality), and, according to the Registrar General's analysis, about half these were a direct result of infarction of the brain. The morbidity associated with this disease is also formidable; death may be preceded by months or even years of mental deterioration or paralysis.

For more than a century autopsies on cases of cerebral infarction have revealed frequently an unexpected absence of significant lesions in the local cerebral arteries, and attention has periodically been directed to the possible role of the carotid arteries in this condition. In a study by Dr. Peter Yates and Dr. E. F. Hutchinson on behalf of the Medical Research Council, 100 cases of suspected cerebral ischæmia were investigated in detail *post mortem*, and in 35 of these cerebral infarction was found*. The whole course of the cerebrovascular tree from the aorta to the brain was examined, the vertebral as well as the carotid arteries being dissected, and the pathological findings were correlated with clinical records; Dr. Yates was mainly concerned with the pathological side of the work, and Dr. Hutchinson with the clinical aspects.

Of the cases of cerebral infarction, little more than half showed occlusion or significant narrowing of the local arteries, but serious obstructions of the extracranial cerebral arteries was found in 90 per cent. Such a high frequency of extracranial arterial obstruction in cases of cerebral infarction suggests that surgery may have a wider application in the early stages of cerebrovascular disease than has been generally recognized.

The authors emphasize, however, that cerebral infarction rarely has a single cause, and that it is usually the result both of obstruction in extracranial or intracranial cerebral arteries and of systemic disease. The decisive part played by general systemic factors, especially those that result in a lowering of the blood pressure, is amply shown in this report. Even when cerebral arteries, whether extracranial or intracranial, are grossly narrowed, the blood circulation in the brain may be maintained for long periods, provided that there is an adequate collateral supply; but if hypertension or anæmia occurs in addition, the precarious balance may be upset and cerebral infarction precipitated.

* Privy Council. Medical Research Council Special Report Series, No. 300: *Cerebral Infarction—The Role of Stenosis of the Extracranial Cerebral Arteries*. By Peter O. Yates and Edward C. Hutchinson. Pp. viii+95+20 plates. (London: H.M. Stationery Office, 1961.) 14s. net.

STANFORD–PACIFIC NAVAL LABORATORY CONJUGATE POINT EXPERIMENT

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A CLOSE correlation has been discovered in the times of occurrence of very low frequency and extra low frequency electromagnetic phenomena observed at high-latitude conjugate locations. The observations were carried out jointly by the Pacific Naval Laboratory of the Defence Research Board of Canada and the Radioscience Laboratory of Stanford University during January 1961. For this experiment the Pacific Naval Laboratory extended the range of its 0·003–3 c.p.s. investigations of geomagnetic micropulsations to include the natural electromagnetic background up to about 30 c.p.s. Similarly, Stanford very low frequency investigations were broadened to cover from 15,000 c.p.s. down to about 5 c.p.s. Combined observations were made at Byrd Station, Antarctica (80° S., 120° W.), and at two near conjugate locations in the vicinity of Hudson Bay in the Canadian Arctic. The nearest, 200 miles south of the conjugate point on the Vestine model, was at Great Whale River, 55° 17′ N., 77° 46′ W., and the other at Fort Churchill, 58° 42′ N., 94° 03′ W., some 500 miles west of the conjugate point. The geomagnetic co-ordin-

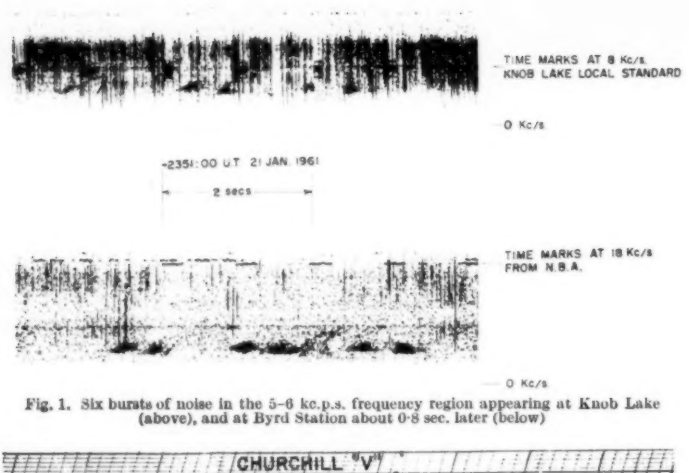

Fig. 1. Six bursts of noise in the 5–6 kc.p.s. frequency region appearing at Knob Lake (above), and at Byrd Station about 0·8 sec. later (below)

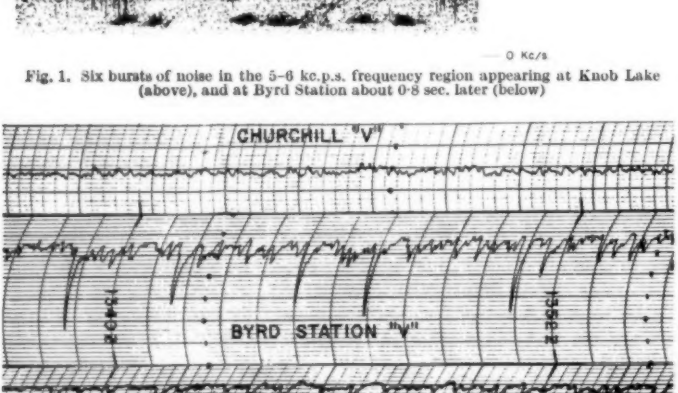

Fig. 2. Envelopes of the activity received on the 2–30 c.p.s. (V) system at three auroral stations. The record for Churchill is in inverted relation to the other two

ates of these stations are 70·4° S., 337·5° E.; 67·8° N., 350° E.; and 68·7° N., 322·8° E., respectively.

Stanford very low frequency equipment consisted essentially of a vertical single-turn loop antenna with an effective area of 80 square metres, coupled to a broad-band audio-frequency amplifier[1]. The signals were recorded on magnetic tape. Observations were made at synoptic intervals of 2 min. each hour so that later comparison could be made with data from the various stations of the Whistlers-East and Whistlers-West networks. Of particular interest in this regard were the observations made at Knob Lake, Canada, 67·5° N., 1° E. geomagnetic, some 400 miles south-east of the Vestine point conjugate to Byrd Station. These records were kindly loaned by Dr. M. G. Morgan. Observations taken at points along the magnetic meridian passing through Byrd Station (very low frequency traverse) were also available.

Pacific Naval Laboratory equipment[2] at the conjugate stations consisted of multi-turn mumetal strip-cored detectors oriented to measure the geomagnetic fluctuations between 0·003 and 3 c.p.s. in the conventional X, Y and Z geographical axes. At pre-arranged times the X, Y and Z detectors at these stations were sharply tuned to one of four frequencies (0·8, 1·1, 1·6 or 2·2 c.p.s.). Single components were monitored at Ottawa and Victoria. In addition, signals in the 2–30 c.p.s. bands were received on horizontal two-turn loops of 3,000 ft. circumference. The X, Y and Z outputs and the envelope of the 2–30 c.p.s. band were continuously recorded on Esterline-Angus charts, and magnetic tape records were made at pre-arranged intervals.

Analysis of synoptic very low frequency records taken at Byrd Station and Knob Lake has shown that various strong bands of very low frequency emission activity in the 200–1,200 c.p.s. range are often present at the same times. Table 1 shows the correlation coefficients versus frequency calculated from simultaneous intensity measurements. It was found that the emission bands showing a distinct lower frequency cut-off at Byrd Station appeared more frequently at Knob Lake than those bands showing no distinct lower border. Discrete bands of very low frequency emissions were also often closely related. Fig. 1 shows a sample of the close correlation in time and character of noise bursts at about 5 kc.p.s. as observed at Byrd Station and Knob Lake. The emissions shown arrive at Knob Lake 0·8 ± 0·1 sec. prior to their appearance at Byrd Station. Timing at both Byrd and Knob Lake was measured independently, utilizing the time pips from Station *NBA* so that the only significant limitation on the accuracy of the measurements is the lack of definition of the emissions themselves. (Typical whistler time delays at 5 kc.p.s. for one traverse of the path at these latitudes lie between 1·5 and 2·5 sec.)

Signals recorded in the 2–30 c.p.s. range also show close conjugate correlation. Fig. 2 shows a comparison of the envelopes taken at Byrd, Great Whale and Churchill. It can be seen that the first two have a higher degree of correlation, not only of the bursts in amplitude, but also in much of the detailed structure between the bursts.

Although only a few magnetic tapes of these extra low frequency signals have been analysed to determine the spectral composition, it seems that four distinct and independent bands of noise are often present. These bands are usually centred about 8, 14 and 20 c.p.s., and one, more variable than the others, centred between 25 and 30 c.p.s. The bands centred about 8 c.p.s. and 14 c.p.s. predominate (Fig. 3) and are found at all three auroral stations. The amplitude bursts in Fig. 2 are associated with enhancements in the lower frequency bands, usually 8 c.p.s. It seems probable that each band is made

Table 1. Spearman Rank Correlation Coefficient versus Frequency for 48 Consecutive Hourly Samples of Very Low Frequency Noise at Byrd and Knob Lake

Frequency in c.p.s.	200	300	400	500	700	1,000	2,000	3,000	4,500	7,000	10,000
Correlation coefficient	+0·46	+0·48	+0·51	+0·59	+0·48	+0·52	+0·14	+0·04	+0·26	+0·30	+0·17

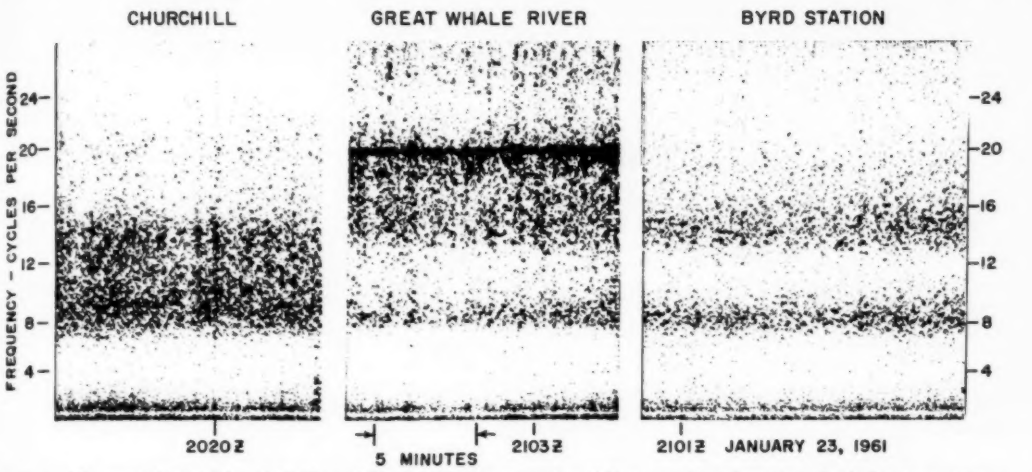

Fig. 3. The spectral distribution of signals received by the 2–30 c.p.s. system at three auroral stations at nearly similar times. The narrow band near 20 c.p.s. at Great Whale River is of undetermined origin

up of a multitude of discrete signals, similar to 'chorus' in the very low frequency regions. In the frequency-range below 30 c.p.s. world-wide lightning is a chief source of noise received on a vertical loop. However, lightning does not appear to contribute substantially to signals received on the horizontal loop.

In the micropulsation-range (0·02–3 c.p.s.), although activity at the auroral stations was significantly more complex, during undisturbed times all stations often showed a marked similarity. Enhancements of activity accompanied by visible auroræ usually showed a closer relationship in time and character between Great Whale River and its near conjugate at Byrd Station than between any other pair. Auroræ were, of course, not visible in the antarctic summer, but from the character of the micropulsation records they were probably present. Enhancements due to geomagnetic activity associated with sudden increase on ordinary magnetograms were received by the 2–30 c.p.s. equipment on several occasions. These were generally due to frequencies well below 2 c.p.s. which were of large amplitude and usually coincident within 2 min. at Byrd Station and Great Whale, while their occurrence and timing were often more independent at Churchill.

It seems that the similarity of records taken at Great Whale and Byrd results from their close conjugate relationship. This view is supported by the generally lower correlation of Churchill with Great Whale and Byrd. Very low frequency observations taken in the Antarctic show little correlation of activity below 3 kc.p.s. at stations 1,200 km. apart (Byrd Station and South Pole Station), and results from the very low frequency traverse indicate that at these frequencies significant differences between simultaneous recordings appear at distances of 200 km. Although this suggests that much of the very low frequency emission activity observed in the auroral regions may be initiated in the lower ionosphere, it does not exclude the possibility that these emissions may be generated at a much greater height. The small time difference between the occurrence of identical events at opposite ends of a field line suggests that these events may result from particles impinging on the Earth's magnetic field not far from the equatorial plane.

This work was supported in part by National Science Foundation grant 13532 and partly by the Defence Research Board of Canada under Project *D*45-95-11-39.

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INTERPLANETARY DUST NEAR THE EARTH*

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THE problem of gravitational accretion of interplanetary dust[1–3] by a planet has recently been the subject of renewed attention. In particular, the possibility of observing dust impacts experimentally at various distances from the centre of the Earth by means of rockets, satellites and space probes has stimulated an inquiry into the concentration of dust around the Earth[4,5]. Beard[4] has predicted that the dust concentration should increase as the inverse 3/2 power of distance, that is, as $r^{-3/2}$, and by a factor of 10^3 over the value in interplanetary space. Whipple[6] has recently published a compilation of rocket and satellite data which exhibits a rapid increase of counting-rate as the Earth is approached, by about

* Substance of a paper at the Spring Meeting of the American Geophysical Union, Washington, D.C., April 1961.

10^5. He identifies this phenomenon with the existence of a permanent 'dust belt' around the Earth.

The purpose of the present article is to point out that a more complete theory does not lead to a continuous increase in dust concentration as Earth is approached, but rather to a maximum at about 2,000–3,000 km. above sea-level, giving rise to a 'dust shell'. The increase amounts to a few times the value of the concentration of dust in interplanetary space, rather than a factor of 1,000 as given by Beard[4,7]. However, since detectors do not measure concentration, but instead count particles having a certain momentum above a minimum value, the expected increase in counting-rate as one approaches the Earth is in fact larger, but still not in accord with Whipple's compilation. We are therefore left with the conclusion: (1) that other factors besides gravitational enhancement take a role in a proper theory, for example, particles in captured orbits[5,6,8]; or (2) that the variations with time in the intensity of the dust particles are very strong so that this effect must be taken into account in assembling a compilation.

First I will neglect all forces except gravitational forces; hence, the results given here apply to large dust particles, certainly for dust particles of the order of 100 microns. (For smaller dust particles radiation pressure forces must be considered, as well as force arising from the electric charge of the particle, notably the Lorentz force and Coulomb drag[3].) It is assumed that the geocentric velocities of these dust particles are isotropically distributed. This means that at large distances from the Earth, where the gravitational potential due to it is negligible, the particles have randomly oriented velocities with respect to the Earth. Furthermore, these velocities are small, of the order of a few km. per sec. This conclusion is arrived at by considering the action of the Poynting–Robertson effect on the orbits of small dust particles[2,9].

The following notation is used: distances r are measured in terms of R, the radius of the planet, denoting $y = R/r$; velocities are measured in terms of $v_\infty = (2GM/R)^{1/2}$, the escape velocity from the surface of the planet. The energy ratio (GMm/R) to $(\frac{1}{2}mu^2)$, where u is the (geocentric) velocity at infinity of the particle, will be denoted by E. Thus:

$$E = 2GM/Ru^2 = v_\infty^2/u^2 \tag{1}$$

From conservation of energy it follows that:

$$\tfrac{1}{2}mv^2 = \tfrac{1}{2}mu^2 + GMm/r \tag{2}$$

Hence:

$$v^2 = u^2 + v_\infty^2\, y \tag{3}$$

At a level determined by r, the speed of the particle is defined by equation (3). The allowed directions are defined as follows at any point r: (i) particles incident with zenith angle θ between 0 and a certain value θ_c become accreted by the planet and therefore contribute to the density only when moving towards the planet; (ii) particles incident with θ between θ_c and $\pi/2$ return again to infinity and therefore contribute twice.

The angle θ_c corresponds to the trajectory which grazes the surface of the planet. Hence, by conservation of angular momentum:

$$rv \sin\theta_c = Rv_R \tag{4}$$

where

$$v_R^2 = u^2 + v_\infty^2$$

Hence:

$$\sin^2\theta_c = y^2\,\frac{u^2 + v_\infty^2}{u^2 + v_\infty^2\, y} \tag{5}$$

I now make the reasonable assumption that the geocentric velocities u of the particles are isotropically distributed, that is, they are at large distances from the Earth. I also take a unique value of u, although my approach can be generalized in a straightforward manner to deal with a distribution of velocity values.

Since by Liouville's theorem the phase space density D remains constant, the density ρ at r will be given by:

$$\rho(r) = c\Big[\int_0^{\theta_c} v \sin\theta\, d\theta + 2\int_{\theta_c}^{\pi/2} v \sin\theta\, d\theta\Big]$$
$$= C(u^2 + v_\infty^2\, y)^{1/2}\,(1 + \cos\theta_c) \tag{6}$$

With the help of equation (5):

$$\rho(r) = C(u^2 + v_\infty^2\, y)^{1/2}\left[1 + \left(1 - y^2\,\frac{u^2 + v_\infty^2}{u^2 + v_\infty^2 y}\right)^{1/2}\right] \tag{7}$$

The normalization constant is obtained by setting $y = 0$:

$$\rho_\infty = 2Cu$$

If all velocities are now expressed in terms of v_∞, then:

$$\frac{\rho(y)}{\rho_\infty} = \left(\frac{u^2 + y}{4u^2}\right)^{1/2}\left[1 + \left(1 - y^2\,\frac{u^2 + 1}{u^2 + y}\right)^{1/2}\right] \tag{8}$$

The function is plotted in Fig. 1 and shows some extremely interesting features. In the first place, it is seen that the density in cislunar space, in particular close to the Earth, is always enhanced over the density in interplanetary space. The enhancement is particularly pronounced for small values of u. The remarkable feature, however, is the fact that a pronounced peak exists always above the atmosphere.

When u exceeds $3^{-1/2}$ the density ρ_R very close to the Earth drops below the free-space density. This can be seen more clearly by setting $y = 1$ in equation (8). Hence:

$$\rho_R/\rho_\infty = [(u^2 + 1)/4u^2]^{1/2} \tag{9}$$

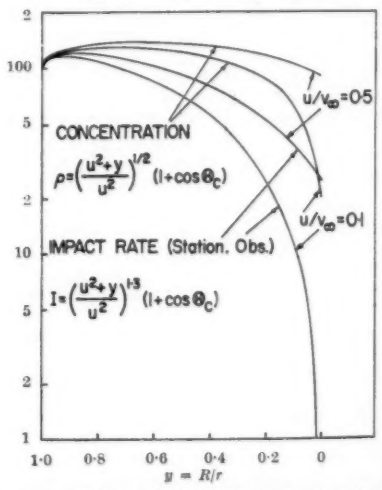

Fig. 1. Concentration of zodiacal dust as a function of distance from the centre of the Earth. Also plotted is the impact-rate which would be observed by an instrument moving at very low speed with respect to the Earth (case 1). The graphs convey the spread to be expected with geocentric velocities of the zodiacal dust in interplanetary space of about 1–5 km. per sec.

which for small values of u reduces to $(2u)^{-1}$ and for large values of u to 1/2.

For small values of u also equation (8) can be replaced by:

$$\rho(y)/\rho_\infty = (2u)^{-1}\, y^{1/2}\,[1 + (1 - y)^{1/2}] = \rho_R\, y^{1/2}\,[1 + (1 - y)^{1/2}] \qquad (10)$$

We can investigate the remainder of the expression (10). It reduces to 1 at $y = 1$. If we replace y by $1 - \varepsilon$, then the expression reduces to $1 + \varepsilon^{1/2}$. Therefore, we see that there is always a peak below $y = 1$, and therefore at some altitude above sea-level. This peak is produced by a 'shadow effect' of the planet.

The rate at which dust particles are accreted by the Earth can also be investigated; this rate is given by:

$$\rho_\infty\ (\pi R^2)\ u\ (1 + v_\infty^2/u^2) \qquad (11)$$

The expression is seen to have a minimum value when the geocentric velocity u equals the sea-level escape velocity v_∞ (= 11·2 km./sec. for the Earth). From the equation of continuity we can also deduce the mean radial velocity near sea-level. It is equal to equation (11) divided by ρ_R. Using equation (9) we obtain:

$$v_{\text{rad}} = 1/2\ (u^2 + v_\infty^2)^{1/2} \qquad (12)$$

Comparison with Observations

We can use the results of the preceding section to calculate two quantities which can be checked by observations: (1) the absolute value of the rate of accretion by the Earth; (2) the relative counting-rate of a meteor impact detector as a function of distance from the Earth (the absolute rate depends on the sensitivity of a particular detector).

(1) *Accretion.* A normalization must be made to the density of interplanetary dust in the vicinity of the Earth's orbit. Photometric investigations based on measurements of the zodiacal light give values for ρ_∞ which vary in the range of 2×10^{-22} to 5×10^{-21} gm./cm.^{3} (refs. 10 and 11). Using this range of values in eq. (11), Table 1 is obtained:

Table 1. Accretion of Interplanetary Dust to the Earth (in tons per day)

ρ_∞ (gm./cm.^{3})	u/v_∞ = 0·1	0·2	0·5	1·0
2×10^{-22}	2,450	1,260	605	485
5×10^{-21}	$6{\cdot}1 \times 10^4$	$3{\cdot}1 \times 10^4$	$1{\cdot}5 \times 10^4$	$1{\cdot}2 \times 10^4$

An estimate for the minimum value of u/v_∞ can be obtained by assuming the heliocentric orbits of the dust particles to be completely circular and inclined to the ecliptic at an angle of about 2°, a mean value for the nearer planets. The Earth's orbital velocity is 29·8 km./sec., giving a geocentric velocity of the dust particles of more than 1 km./sec. Again, since the eccentricity of the Earth's orbit is 0·0176, the perihelion to aphelion velocity ratio turns out to be $1 + \varepsilon/(1 - \varepsilon)$, or about 3 per cent of the orbital velocity. We can therefore take $u = 1$ km./sec. as a lower limit for the geocentric velocity of interplanetary dust, with most of the dust component responsible for the zodiacal light in the range 1–5 km./sec. ($u/v_\infty \sim 0{\cdot}1$–$0{\cdot}5$).

It can then be seen from Table 1 that the lower limit of ρ_∞ of about 2×10^{-22} gm./cm.^{3} gives an accretion-rate in accord with the generally quoted value of about 1,000 tons per day.

(2) *Rate of impact.* Before any comparison is made with observations, it must be realized that it is never the concentration, given by equation (8), which is measured. Generally, an acoustic detector counts the number of particles which impart a momentum greater than some minimum value p_m.

But assuming (1) that detectors have similar values of p_m, and (2) that the momentum of impact is proportional to particle momentum (at least over the range of velocities encountered here), we can calculate the expected counting-rate vs. distance. (Actually, the results are fairly insensitive to assumption (1), see ref. 12.) However, it is quite important to bring in another factor, namely, the velocity of the vehicle on which the detector is mounted.

I have therefore considered three cases[12]: Case 1, stationary observer, that is, a rocket near peak altitude; Case 2, a space probe moving vertically outward with local escape velocity; Case 3, a satellite in circular orbit.

The results for Case 1 are shown in Fig. 1, using a dust particle cloud the particle-radii of which follow the law $f(a)da = a^{-2.5}\,da$. It can be seen that a peak still exists for the rate of impact but at a lower altitude than for concentration. However, the total variation of impact-rate is much greater: between infinity and the vicinity of the planet it increases between a factor of 4 (for $u/v_\infty = 0{\cdot}5$) up to a factor 200 (for $u/v_\infty = 0{\cdot}1$).

Conclusions

My results predict the existence of a modest dust shell around the Earth, instead of a variation with a factor of 10^3–10^5 increasing uniformly without a maximum[4,6].

Experimental evidence concerning a concentration of interplanetary dust in the vicinity of the Earth is as yet very scanty. The data come from different detectors, different types of vehicles, namely, rockets, satellites, and space probes (corresponding to the three cases mentioned), and were obtained at different times[13]. Discrepancies among individual measurements amount to orders of magnitude at times[14], indicating the possibility of real variations with time, due perhaps to a non-zodiacal dust component.

It appears to be premature, therefore, to draw any firm conclusions from the experimental data at present available about the existence of a dust shell of the type discussed here. Nevertheless, a confirmation of its presence with a maximum as shown, for example by Fig. 1, would be of great importance in establishing the validity of the assumptions underlying the theory of the present work.

I thank M. Dubin, E. J. Öpik and C. W. McCracken for illuminating discussions. This work was supported by a grant of the National Aeronautics and Space Administration, *NsG*-58-60.

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NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF PROTEINS: THE HYDROGEN BONDING OF WATER IN BOVINE SERUM ALBUMIN SOLUTIONS

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THE nature of the 'water of hydration' in aqueous protein solutions has been the subject of much investigation and discussion[1–4], but there is little direct experimental evidence as to whether it exists in a 'frozen' form, comparable with ordinary ice[3], or in some less highly organized state. It appeared that measurement of the nuclear magnetic resonance peak positions of water protons in concentrated protein solutions might furnish some information on this point. In this communication results are presented for bovine serum albumin solutions at 25° and *p*H 6·8.

Arnold and Packard[5] have observed that the chemical shift of the hydroxyl proton signal of ethanol is dependent on temperature, moving upfield by about 1·5 p.p.m. (with respect to the methylene signal) over the temperature range −117° to 78°. This was believed to arise from the breaking of hydrogen bonds[6]. Similar shifts have been observed on dilution of alcohols[7–9] and phenols[10,11] with non-polar solvents and have been satisfactorily interpreted as due to the dissociation of hydrogen-bonded polymers.

Cohen and Reid[7] observed that at 29° the peak position for water dissolved in carbon tetrachloride is at a field 3·68 p.p.m. higher (after bulk magnetic susceptibility correction) than for liquid water. Schneider, Bernstein, and Pople[12] found that for water vapour the peak position is 4·58 p.p.m. higher than for liquid water at 0°, susceptibility correction being made. Very recently, Tiers and Kowalsky[13] have found that the peak position for liquid water, measured with respect to the methyl group resonance of dissolved sodium 2,2-dimethyl-2-silapentane-5-sulphonate as internal reference (assigned a scale value $\tau' = 10{\cdot}000$), moves to higher field with increasing temperature. At 0°, $\tau' = 4{\cdot}983$; at 25°, $\tau' = 5{\cdot}219$. The peak position reported by Cohen and Reid[7] for water in carbon tetrachloride corresponds to 8·93 on the τ' scale. The vapour value of Schneider *et al.*[12] is approximately 9·56 on the τ' scale, but to be useful this value requires a rather large negative correction for 'solvent shift'[14], which appears to take place for all substances on passing from the vapour to the liquid state, whether hydrogen-bonded or not. Since the magnitude of this correction for water is unknown, the value 8·93 τ', based on water in carbon tetrachloride, has been chosen as representing the best approximation to the resonance position of water protons when all hydrogen bonds are broken. Assuming, with Pauling[15], that in ice every proton is involved in a hydrogen bond and that in liquid water at 0°, 15 per cent of these bonds are broken, I calculate that for completely hydrogen-bonded liquid water, if it were capable of existence, we would observe the proton resonance at 4·23 τ'. We thus have a total difference of chemical shift of 4·70 p.p.m. between hydrogen-bonded and non-hydrogen-bonded protons in water, and since peak positions are reproducible within ± 0·005 p.p.m. in the present work (see below), a change in hydrogen-bonding involving 1 per cent of the water protons should be measurable, while changes involving only a few tenths of 1 per cent should be easily detectable.

A 'Varian *V*-4300–2' 40·00-Mc. per sec. spectrograph was used, together with a 'Varian' variable-temperature probe, sample spinner, audio-oscillator, Hewlett–Packard '522-*B*' frequency counter and 'Varian' recorder. Approximately 0·5 ml. samples of protein solutions were used, using 5 mm. (outside diameter) 'Pyrex' tubes. Samples were maintained at 25·0 ± 0·2°; maintenance of a constant temperature is particularly important when external referencing is used, as described later. All tubes were evacuated and freed of air by successive freezing and thawing; they were then sealed under 0·5 atmosphere of pre-purified nitrogen.

The bovine serum albumin used was purchased from Pentex, Inc. (Kankakee, Ill.) and was designated 'Lot 9*G*–10'; it was found to contain 3·5 per cent moisture. Solutions were prepared in deionized water. The *p*H of all solutions was found to be 6·80 ± 0·05; no buffers or salts were added.

For measurement of water peak positions, internal referencing was first attempted. It was found that, measured with respect to sodium 2,2-dimethyl-2-silapentane-5-sulphonate (2 per cent)[13], the water peak of an 11·9 per cent bovine serum albumin solution shifted −0·102 p.p.m. compared with pure water. It was also noted that the reference peak, normally narrow and prominent, became increasingly broad at higher protein concentrations, being barely observable in the 11·9 per cent solution and unobservable in the 23·9 per cent solution. This appeared to indicate that the sulphonic acid anion was fairly strongly adsorbed by the protein and was experiencing the latter's much longer correlation time[16]. It therefore could not be regarded as sampling all regions of the solution indifferently, as a reliable internal reference should. It was found that 1 per cent of 2,2-dimethyl-2-silapropanol-1 (ref. 13) showed much less but still perceptible broadening of the methyl peak and gave also a smaller apparent shift, − 0·072, of the water peak in the 11·9 per cent solution. *Tert.*-butanol (2 per cent), which is much more soluble than the silapropanol, showed no broadening and an apparent shift of water peak of only − 0·019 in 11·9 per cent protein solution. To avoid any suspicion of spurious shifts, however, it seemed best to rely chiefly on an external reference. For this purpose, hexamethyldisiloxane (obtained from the Dow-Corning Corp.) was sealed into 1 mm. (internal diameter) capillaries, which were then inserted into the 5 mm. nuclear magnetic resonance sample tubes. Peak positions were measured by the 'frequency changing' method already described by Tiers[17]. At least six measurements were made on each system; values obtained on increasing and decreasing sweeps were

averaged to eliminate systematic error from this source. Measurements were found to be reproducible within ± 0·005 p.p.m. average deviation at most; usually the limits were narrower. Only differences in peak positions between water and protein solutions were of interest, and therefore no correction for difference of bulk diamagnetic susceptibility between solvent and reference was necessary.

Solutions of methylamine hydrochloride, potassium acetate, and potassium butyrate were prepared from reagent or C.P. materials and their *p*H values were adjusted to within ± 0·01 unit using a 'Radiometer' *p*H meter. The nuclear magnetic resonance tubes were sealed under purified nitrogen in the same manner as the protein solutions.

Water proton peak positions were measured at 25·0° with respect to hexamethyldisiloxane as external reference for pure water and for solutions containing 0·030, 0·060, 0·119 and 0·239 gm. of bovine serum albumin per c.c. of solution (W_2). There was an appreciable broadening of the signal at higher protein concentrations (up to approximately 5 c.p.s. half-height width at $W_2 = 0{\cdot}239$), but this did not impair the measurements. The observed differences in peak position are shown in the third column of Table 1, in which $\Delta\delta(\delta_{\text{water, protein sol.}} - \delta_{\text{water}})$ is recorded. Because the diamagnetic susceptibility of the protein solution differs somewhat from that of water, a correction must be made when employing an external reference. The volume susceptibility χ_s of the protein solution can be calculated from:

$$\chi_s = W_1\chi_1 + W_2\chi_2$$

where χ_1 and χ_2 are the gram susceptibilities of water and (dissolved) protein, respectively. We have taken χ_1 as $-0{\cdot}721 \times 10^{-6}$ (ref. 18*a*) and have calculated χ_2 for bovine serum albumin from the Pascal constants and constitutive corrections, as listed by Pople, Bernstein and Schneider[18*b*]. In column four of Table 1 the calculated values of χ_s are listed. From these the value of the corrected shift of the water peak, $\Delta\delta_{\text{corr.}}$ in column 5 is calculated, using the relationship[19]:

$$\Delta\delta_{\text{corr}} = \Delta\delta_{\text{obs}} + \frac{2\pi}{3}(\chi_1 - \chi_s)$$

It can be seen that, within experimental error, the differing magnetic susceptibility is entirely responsible for the apparent shift in the water peak of the protein solution.

In the most concentrated bovine serum albumin solution ($W_2 = 0{\cdot}239$), it can be calculated that the carboxylate group concentration is approximately 0·38 *M*, the ammonium and guanidinium group concentration (not considered separately) approximately 0·32 *M*, and the peptide group concentration approximately 2·2 *M*. Direct measurement at *p*H 6·8 of effect of methylammonium ion (0·5 *M*), acetate and butyrate ion (1 *M*), and *N*-methylacetamide (2·5 *M*), as models for these groups, showed that in the protein solutions they would not affect the position of the water peak within the experimental error of measurement.

It appears that even in very concentrated bovine serum albumin solutions there is no change in the hydrogen-bonding of the water molecules which involves more than 0·1–0·2 per cent of the water protons. By 'change' is meant a disruption of the hydrogen bonds normally existing in water, or the formation of new additional hydrogen bonds (see below) whether with other water molecules or with the protein.

Table 1. Observed and Corrected Relative Proton Peak Positions for Water in Solutions of Bovine Serum Albumin (BSA); 25·0°

BSA conc., gm./c.c. solution (W_2)	Water conc., gm./c.c. solution (W_1)	$\Delta\delta_{\text{obs.}}$	$-\chi_s \times 10^6$	$\Delta\delta_{\text{corr.}}$
0	1·000	0	0·721 (= χ_1)	—
0·030	0·978	−0·006	0·724	+0·002
0·060	0·955	−0·008	0·727	+0·006
0·119	0·912	−0·031	0·733	−0·004
0·239	0·821	−0·060	0·746	−0·006

Let us first suppose (somewhat naïvely) that protein molecules, on being introduced into water, have no effect on the structure of the water other than to interrupt it at the protein–water interface. Taking the bovine serum albumin molecule as a cylinder 145 Å. in length and about 30 Å. in mean diameter[20], it can be estimated that about each there will be a monolayer containing at least 1,000 water molecules, corresponding to about 28 per cent 'hydration'. Assume that each interfacial water molecule suffers the breakage of one hydrogen bond. In a solution for which $W_2 = 0{\cdot}24$ and $W_1 = 0{\cdot}82$ (Table 1), it is readily calculated, assuming a molecular weight of 65,000 for the protein, that about 5 per cent of the total hydrogen bonds of the water would be broken. This should produce a $\Delta\delta$ of + 0·235, exceeding the experimental error by fifty-fold. It seems, then, that the interruption of the water structure must be compensated by the formation of other hydrogen bonds, either to the peptide linkages or other polar groups of the protein, or in some other way. We have seen that the interaction of water molecules with carboxylate, amino, and peptide linkages in the concentration present in such a protein solution has an essentially negligible effect on the water resonance position, so that the replacement of water–water hydrogen bonds by bonds to these groups is not directly seen as such.

In addition to forming hydrogen bonds with the protein, the interfacial water molecules may become re-arranged in such a way as to form bonds among themselves to the same degree as in water alone, despite the interrupting interfaces. It has been recognized for some time[21] that when hydrocarbons are dissolved in water the heat effect is often slightly negative but that there is a large decrease in entropy, indicating a clustering of water molecules about the intruding hydrocarbon but no breakage of hydrogen bonds. These observations have been recently reviewed and their significance for protein structure pointed out by Kauzmann[4]. It is not unreasonable to suppose that a similar clustering of water molecules occurs about the hydrophobic portions of dissolved proteins (at least 40 per cent of the amino-acid residues of bovine serum albumin can be regarded as hydrophobic); the present results appear to be consistent with this supposition.

Kauzmann has pointed out[4] that the 'icebergs' presumed to form about hydrophobic areas of the protein should not be identified with the 'frozen water' which has been thought by several authors to exist around proteins, and to which a very important part in protein structure and function has been assigned by Klotz[3]. The hydrophobic icebergs have in fact a relatively low thermodynamic stability and would tend to lead to aggregation and insolubility. The nature of this 'ordered' or 'frozen' water, which presumably constitutes the major share of the water of hydration, is still in doubt. The evidence presented here indicates that this ordered water cannot be

regarded as having a structure like that of ice (a conclusion also indicated by other lines of evidence). If we suppose that the water of hydration (taken as 0·28 gm. per gm. protein) has an ice-like structure and that its protons exchange rapidly with the surrounding water, I expect that in the bovine serum albumin solution in which $W_2 = 0{\cdot}24$, a $\Delta\delta$ of approximately $-0{\cdot}067$ would be observed, compared with pure water, which again is well outside my experimental precision. It is possible, of course, that there is a disruption of water hydrogen bonds near hydrophobic areas of the protein, and that this is just compensated by more extensive bonding in the 'ordered' or 'frozen' portions of the hydrate water. But it appears more logical at present to regard the present results as supporting Kauzmann's conclusions concerning the nature of the water in the hydrophobic areas, and to regard the remainder of the hydrate water as having a degree of hydrogen bonding equal to that of pure water.

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PURIFICATION OF URINARY CONJUGATED ŒSTROGENS BY GEL FILTRATION

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A GEL filtration method for de-salting and group-separation of water-soluble substances has recently been worked out by Porath and Flodin[1]. This method is based on a column procedure similar to chromatography in which the stationary phase is comprised of a new type of gel ('Sephadex', manufactured by Pharmacia, Ltd., Sweden). The present report suggests that this technique might be of use for the purification and group-separation of conjugated œstrogens in pregnancy urine. It might also form the basis of a simple assay method for determination of œstrogens in pregnancy urine.

In the early experiments a solution of 5 μgm. of sodium œstriol glucusiduronate (Sigma, Ltd., U.S.A.) and/or sodium œstrone sulphate (Schering, Ltd., Germany) in 1 ml. of 0·15 *M* acetate buffer (*p*H 7·0) was applied directly to a 1 × 50 cm. 'Sephadex' (*G*25) column. As soon as this solution was percolated into the gel, elution was performed with distilled water. One-ml. fractions of the effluent were collected and, after evaporation of the tubes, the amount of œstrogen present in each fraction was measured by a modified Kober reaction[2]. In these experiments the two conjugated œstrogens were eluted in one distinct peak, which was invariably found in fractions 40–43. Other conjugated œstrogens, such as the sodium salts of 17β-œstradiol-17-glucusiduronate, 17β-œstradiol-3-sulphate, 17β-œstradiol-17-sulphate, œstriol-3-sulphate, 17β-œstradiol-3-phosphate and 17β-œstradiol-17-phosphate, were also eluted in fractions 40–43.

In the next series of experiments the conjugated œstrogens listed above were added to male urine which was then percolated through the 'Sephadex' column. Usually a concentration of 2–5 μgm. of conjugated œstrogen per ml. of urine was used. 1–20 ml. of these urine samples were applied to the columns without previous purification. As soon as the urine was completely percolated into the bed material, elution was performed with distilled water and 1-ml. fractions of the effluent were collected. Since the main part of urinary pigments was eluted in fractions 22–36, it was not possible to apply a direct Kober reaction to these tubes. Therefore the individual fractions were incubated with an extract of the digestive tract of the snail (*Helix*

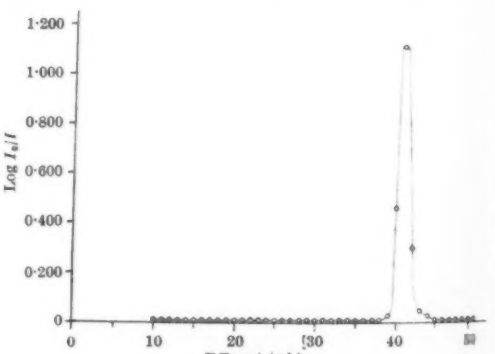

Fig. 1. Elution curve obtained by gel filtration of 2 ml. of male urine containing 10 μgm. of sodium œstriol glucusiduronate. 'Sephadex' (*G*25) column 1×50 cm.; eluant: distilled water; running time: 4 hr.

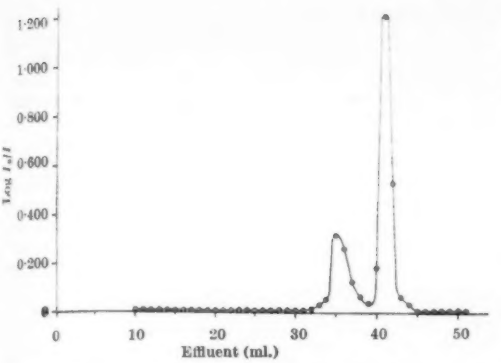

Fig. 2. Elution curve obtained by gel filtration of 1 ml. of pregnancy urine; experimental conditions as in Fig. 1

pomatia, purchased from L'Industrie Biologique Française) and, following enzyme hydrolysis, each fraction was extracted with ether. The ether was evaporated and the amount of œstrogen liberated was measured by the Kober reaction. Under these experimental conditions all the conjugated œstrogens already mentioned added to male urine were eluted in one single zone (fractions 40–43) as shown in Fig. 1.

Approximately 90 per cent of the conjugated œstrogens added to male urine could be recovered within this zone. Also in those experiments where as much as 20 ml. of the urine samples were applied to the columns, all the Kober-positive material was found to be concentrated to fractions 40–43.

On the other hand, when late-pregnancy urine was processed under the same conditions, two distinct peaks were found; one in fractions 33–36 and the other in fractions 40–43 (Fig. 2).

As judged from the elution curve, the second peak of pregnancy urine corresponds to the single peak (Fig. 1) obtained from male urine containing one or more of the reference compounds already named of conjugated œstrogens. It was also found that the addition of small amounts of œstriol glucusiduronate and œstriol sulphate to late pregnancy urine (5–10 μgm. per ml.) resulted in a definite enlargement of the second peak of the elution curve. About 90 per cent of the Kober-positive material present in the two peaks obtained from pregnancy urine was characterized by the Brown method as œstriol. The predominant conjugate of the second peak was found to be identical with œstriol-16 (17 ?)-glucusiduronate. In preliminary work on the conjugated œstrogen present in the first peak, one main compound was found. This appears to be a form hitherto unknown of conjugated œstriol. In paper-partition chromatography or in countercurrent distribution work this compound was found to be more polar than œstriol glucusiduronate.

The gel-filtration technique seems to be most useful for group-separation and purification of conjugated œstrogens in pregnancy urine. Since the recovery from the columns is almost quantitative, the gel filtration seems to be suitable for the isolation of unknown conjugated œstrogens.

As judged from the high yield obtained after enzyme hydrolysis of the purified fractions, the enzyme inhibitors usually present in urine specimens seem to be removed to a considerable extent by the column procedure. This fact made it possible to develop a quantitative method for the estimation of œstrogen in pregnancy urine. The method is based upon gel filtration and enzyme hydrolysis of the purified fractions containing the Kober-positive material. An additional advantage of the proposed method is that after the elution with distilled water the gel is completely regenerated. Furthermore, the columns can be used daily for several weeks without repacking. Since one technician can easily carry out up to 100 determinations a week, this method seems to provide a suitable means for serial estimations of urinary œstrogen excretion in normal as well as in pathological forms of pregnancy.

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CHROMIUM-51 TAGGING OF THE α-CHAIN OF HUMAN HÆMOGLOBIN

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THE separation of the α- and β-chains of hæmoglobin by elution from 'Amberlite *CG*-50', utilizing a urea gradient at *p*H 1·9, has become an almost routine procedure in the study of the tryptic peptide patterns of hæmoglobin[1]. Work by Pearson[2] and by Ebaugh[3] indicated that $Na_2{}^{51}CrO_4$ preferentially labels the β-chain of human hæmoglobin, and these observations suggested to me a method for determining the degree of cross contamination of one chain by the other when separated on 'Amberlite' columns. Under the conditions of my experiments, however, tagging of the hæmoglobins by chromium-51 was almost exclusively limited to the α-chain of human hæmoglobin *A*.

Hæmoglobin solutions were prepared by standard techniques, following washing of the red cells with normal saline, by lysis with water and toluene. One sample of hæmoglobin was further purified by crystallization from ammonium sulphate solutions following Drabkin's method[4]. Globin preparation was carried out by the technique of Anson and Mirsky[5]. 'Radiochromate' (Abbott) of high specific activity (0·547 mc. chromium-51/ml.; 0·00118 mgm. chromium/ml.) was utilized to tag the various hæmoglobin or globin preparations by any one of several methods. Globin (1 gm./100 ml.) was dissolved in 11·7 per cent formic acid, *p*H 1·4, and approximately 80 μc. of chromium-51 added. Following incubation for 90 min. at 32°, the solution was dialysed against water at 4° until no further chromium-51 was lost. The preparation was found to have bound 13 μc. chromium-51/gm. of globin or $0{\cdot}71 \times 10^{-4}$ mole chromium/mole globin. To

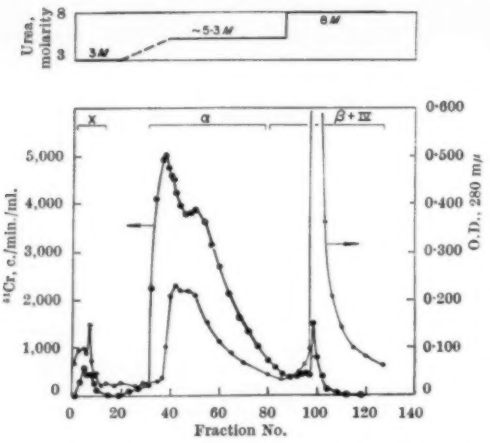

Fig. 1. Separation of α- and β-chains of chromium-51 globin-*A* on '*IRC*-50' Interrupted urea gradient, *p*H 1·9

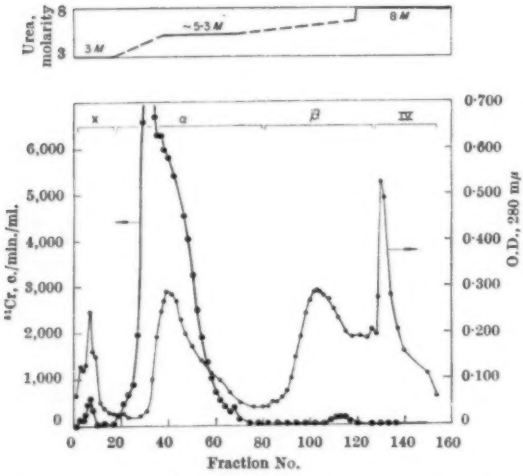

Fig. 2. Separation of α- and β-chains of chromium-51 on '*IRC*-50' Interrupted urea gradient, *p*H 1·9

hæmoglobin solutions (approximately 1 gm./10 ml.) were added the required amounts of dry reagents to constitute a standard acid–citrate–dextrose solution, while purified hæmoglobin powders were dissolved directly into acid–citrate–dextrose solution (approximately 1 gm./10 ml.). In each instance, approximately 75 μc. of chromium-51 were incubated with the hæmoglobin solution for 90 min. at 32°, following which the preparations were dialysed until free of unbound chromium-51. The uptake of chromium-51 was approximately 22 μc. chromium-51 per gram of hæmoglobin or $1·1 \times 10^{-4}$ mole chromium/mole hæmoglobin. Following chromation of the hæmoglobin, globin was prepared from the tagged material by acid–acetone cleavage.

Separation of the α- and β-chains was carried out by my modification of the technique of Wilson and Smith[1] utilizing an interrupted gradient of urea to achieve wider separation of the two chains. This technique, described in detail elsewhere[6], uses an initial wash of the column with 3 *M* urea, until the non-protein peak at 280 mμ has been removed (fraction *X*; Figs. 1 and 2). The progress of elution is automatically monitored at 280 mμ by a direct recording instrument. An increase in the urea gradient is instituted and maintained until the peak of the α-chain has been eluted (approximately 5·2–5·3 *M* urea) at which time the urea concentration is held steady until no further protein can be washed from the column (α fraction; Figs. 1 and 2). The gradient may then be resumed and continued until the second major peak (β fraction; Fig. 2) is eluted before switching to 8 molar urea (to remove the final pigmented material (fraction IV; Fig. 2)) or elution with 8 molar urea may be instituted immediately (Fig. 1). Sample counting was done in a well-type scintillation counter on 3-ml. aliquots of suitably diluted material, and the results recorded as c.p.m./ml. of solution.

The results of two typical experiments are recorded in Figs. 1 and 2. The former experiment involved globin prepared from a hæmoglobin solution, the latter, globin prepared from material purified by ammonium sulphate fractionation. Except for some non-protein bound chromium-51 which was eluted from the column in the first 300 ml., virtually all the chromium-51 appeared in the α peak. Dialysis of this fraction resulted in no loss of chromium-51 activity. Tryptic peptide patterns of the material in the β + IV peak of Fig. 1 showed very slight contamination of the β-fraction with α-chain peptides, while the α-chain digests were essentially free of any peptides specific for the β-chain. No such contamination of the β-chain is suggested in the experiment described in Fig. 2. Total recovery of chromium-51 in this experiment amounted to 96·5 per cent of the material applied to the column. Calculations of the activities recovered in the various fractions from this run showed 1·75 per cent of the chromium-51 in fraction *X*, 95·8 per cent in the α-fraction, 1·95 per cent in the β-fraction and 0·5 per cent in fraction IV (Table 1). Protein recovery and c.p.m./mgm. of protein are also listed in Table 1.

Table 1

Fraction	Protein recovered* (mgm.)	Protein recovered (per cent)	Chromium-51 recovered (per cent)	Activity/mgm. c.p.m./mgm.
X	0	0	1·75	—
α	290	48·4	95·8	4,290
β	213	35·5	1·95	118
IV	107	17·8	0·5	60
Totals	610	101·7	100·00	—

* Protein determinations done by technique of Lowry *et al.* (ref. 7). Starting material, 600 mgm. labelled globin *A*.

Although there seems to be little doubt that, under the conditions utilized in these experiments, chromium-51 is bound to the α-chain, the work of Pearson[2] and of Ebaugh[3] suggests preferential binding to the β-chain of human hæmoglobin *A*. Pearson showed twice the specific activity in hæmoglobin *H* (β_4) compared with hæmoglobin *A* ($\alpha_2\beta_2$) when the hæmoglobins were tagged with chromium-51 and separated at *p*H 7·0 on starch-block electrophoresis. Also, recombination experiments at *p*H 4·7 between chromium-51 tagged hæmoglobin *A* and untagged hæmoglobin *C* yielded no label in the recombined hæmoglobin *C* fraction which, once again, suggested that the chromium-51 was in the β-chain. Ebaugh reports experiments utilizing techniques similar to mine for separating the α- and β-chains of hæmoglobin *A* at *p*H 1·9 in which the label was also found

in the β-chain, although no peptide analyses were done to prove this association. Except for differences in the method of labelling chromium-51, and minor differences in chain separation, the procedures as carried out in our respective laboratories seem comparable. Whether minor differences in *p*H, in mole ratios of chromium to hæmoglobin or in incubation time are of primary importance in determining the site of chromium-51 binding remains to be seen. It is also possible that conditions exist which permit preferential binding of chromium to either chain of hæmoglobin. Work is now in progress to test some of these hypotheses as well as to determine whether chromium-51 binds to a specific site, peptide or amino-acid in the α-chain under the conditions of the experiments reported here.

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EFFECT OF PROGESTERONE ON PREGNANCY

Method of Administration and Effect of Progesterone

THE conclusion that progesterone is a key hormone in the maintenance of pregnancy in both women and laboratory animals has been challenged. It was stressed that the concentration of progesterone in the systemic blood in several species is at its highest value at the onset of labour when myometrial activity and pharmacological reactivity greatly increase, and also that systemic progesterone therapy does not suppress uterine activity in late pregnant women. These observations aided the formulation of the theory of local effect of placental progesterone[1–3].

It was proposed by this theory that placental progesterone affects the myometrium directly, that is, before being carried round by the systemic circulation. Once in the blood-stream the biological potency of progesterone was assumed to be weakened by its 'binding' to some plasma constituents, which in turn affected its 'transport' to target organs. While the local effect of placental progesterone has been demonstrated in a variety of experiments by the functional asymmetry of the uterus, the mechanism by which this effect is enforced remained uncertain.

The experiments to be reported here bring the regulation of progesterone effect from the realm of speculation into that of experimentation, by showing the relative ineffectiveness of intravenous progesterone administration, specifically when the hormone is given in a fat emulsion containing lipids.

New Zealand white rabbits in our colony with a litter size of more than five usually deliver spontaneously on the thirty-second day of pregnancy. However, when induced on the morning of the thirty-first day, by 1 I.U. oxytocin ('Pitocin') intramuscularly, they promptly deliver their uterine contents[4,5]. If induction is carried out on the morning of the thirtieth day, labour is delayed and prolonged in spite of repeated oxytocin treatment, and many placentæ are retained[6]. Thus oxytocin induction is only predictable in the 31-day rabbit, and induced labour is so characteristic that it has been used as a measure of oxytocic potency[5,7].

If 12–24 hr. before induction on the thirty-first day the animal is treated with 5 mgm. progesterone in oil intramuscularly, she does not deliver and remains uninducible so long as progesterone treatment (5 mgm./day in oil, intramuscularly) continues[4,5]. If progesterone treatment is given less than 12 hr. before induction oxytocin is effective on the thirty-first day[4,5]. This and other observations[8] suggest that the progesterone effect has a latency period of 12–24 hr. It is of considerable significance to explain this long period of latency and shorten it if possible.

We have been supplied with a progesterone preparation which has been dissolved in 'Lipomul', a mixture of fat, lecithin, a polymer, and water. This mixture (without progesterone) has been used extensively and in large quantities in human therapy for its high calorie content.

We investigated in rabbits the possibility that intravenous 'Lipomul'-progesterone can shorten the latency period of the progesterone effect when used in massive doses. Thus 40 mgm. progesterone in 10 ml. 'Lipomul' was injected intravenously to pregnant rabbits on the thirty-first day of pregnancy 3 and 6 hr. before induction with oxytocin. The animals promptly responded to oxytocin and delivered as characteristic of the normal 31-day pregnant rabbit. Thus these pilot experiments showed that the latency period is not reduced by massive doses of progesterone (~10 times the effective dose of progesterone, in oil, intramuscularly) injected directly into the systemic circulation.

These negative results prompted the question whether this type of progesterone treatment is effective 12–24 hr. later, for 5 mgm. progesterone dissolved in oil and given intramuscularly is effective. A group of animals received 40 mgm. progesterone in 10 ml. 'Lipomul' intravenously on the thirtieth day and induced 24 hr. later on the thirty-first day. All animals but one delivered as normal untreated rabbits do. The one which failed to deliver on the thirty-first day was successfully induced on the morning of the thirty-second day in spite of the fact that the dose of progesterone was 40 mgm. This failure in progesterone therapy was unexpected in a laboratory where pregnancy in rabbits is regularly maintained by 5 mgm. (or less) progesterone in oil intramuscularly, not only in this type of experiment, but also in the ovariectomized and placental dislocated animal, or after term in the intact animal. We could only account for this failure in progesterone therapy by the route of administration or by the solvent used ('Lipomul').

The difference between the effect of intramuscular and intravenous progesterone was determined by

Table 1

Rabbit No.	Litter size	Total weight (gm.)	Induced delivery on day 31	31	32	32	33	Progesterone administration and solvent
A46	5	257	5					
R211	8	298	8					
M9	9	365	9					40 mgm. in 'Lipomul' (i.v.)
A42	9	418			9			
•*R243*	10	420	10					
•*R215*	7	294	7					
K11	8	430	8					
A16	10	420		10				10 mgm. in oil (i.v.)
A7	9	371	9					
C12	5	276	5					10 mgm. in propylene glycol (i.v.)
I14	12	534	12					4 mgm. in 'Lipomul' (i.m.)
R240	10	386	10					
A47	6	371		6				
R1	9	400				9		
R2	4	196				4		25 mgm. in propylene glycol (i.v.)
WH2	4	157				1	3	
R13	7	357		5	2			
WH20	5	332			5			
G2	8	386					8	100 mgm. in 'Lipomul' (i.v.)
K10	9	307					9	
WH6	9	470			2	7		5 mgm. in propylene glycol (i.m.)
R251	6	278			6			8 mgm. in 'Lipomul' (i.m.)
F15	5	293			5			10 mgm. in 'Lipomul' (i.m.)
K8	10	419					10	40 mgm. in 'Lipomul' (i.m.)

All animals received progesterone treatment on day 30 (except •) and were induced twice a day, starting on the morning of the thirty-first day.
•*R243* and *R215* received progesterone 3 and 6 hr., respectively, before induction on the thirty-first day.

intravenous treatment of a group of 30-day pregnant rabbits with 10 mgm. progesterone dissolved in oil or in propylene glycol. The effect of intramuscular treatment was already known[4,5]. The injections were made through a 26-gauge needle which broke up the oil particles into small droplets. All these animals delivered a normal litter 24 hr. later when induced by oxytocin on the thirty-first day. Thus 10 mgm. progesterone when administered intravenously is ineffective, whereas 5 mgm. intramuscularly is effective.

Using another group of 30-day pregnant rabbits the intravenous dose of progesterone was increased to 25 mgm. (25 mgm. progesterone, dissolved in 0·5 ml. oil, was suspended in 1·5 ml. propylene glycol). These animals did not deliver on the morning or the night of the thirty-first day, but usually on the thirty-second day. Thus 25 mgm. progesterone given intravenously is effective although for only a short period. Thus about five times more progesterone is needed for the same effect when the treatment is intravenous than when it is intramuscular.

If 25 mgm. progesterone is effective when administered intravenously and 40 mgm. 'Lipomul'-progesterone is not, then 'Lipomul' must oppose the progesterone effect. If the dose of progesterone is increased to 100 mgm. the intravenous 'Lipomul'-progesterone becomes effective, in spite of the proportional increase of 'Lipomul' from 10 to 25 ml. Thus the effect of 'Lipomul', inhibiting the exogenous progesterone action, can be overcome by increasing the dose of progesterone.

When only 8–10 mgm. 'Lipomul'-progesterone is given intramuscularly it is effective in defending pregnancy in the 31-day rabbit against oxytocin induction.

What is of interest in these preliminary experiments is the fact that the progesterone effect is greatly dependent on the route of administration and the solvent used. It is known that the *in vitro* effect of progesterone is greatly diminished when the hormone is administered in plasma[6]. It is also demonstrated that the local application of progesterone into the uterine horn is very effective and results in a functionally asymmetrical uterus. As a result of this asymmetry the animal is capable of delivering the contents of the untreated horn while maintaining pregnancy in the treated horn[10]. Recent experiments showed that intramyometrial progesterone treatment is effective in pregnant women (Coutinho *et al.*, and Bengtsson, L. P., personal communications).

Thus problems of progesterone therapy may be partly overcome by discovering methods of administration which permit the ready transport of systemic progesterone to target organs. Such methods may be suggested by experiments on progesterone transport. Studies on the superimposed regulation of steroid effects are important, for we may easily overlook the biological significance of a steroid simply because its concentration in the systemic blood does not fit in with our expectations.

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Effect of a Phospholipid Factor on the Activity of Progesterone

It has been demonstrated that the effect of intravenously administered progesterone is dependent on the solvent used. Pregnancy was prolonged in rabbits by 25 mgm. progesterone in oil–propylene glycol suspension, whereas 40 mgm. progesterone in 'Lipomul' was ineffective (previous communication).

The possibility having been considered that certain compounds in 'Lipomul' counteract the effect of exogenous progesterone in prolonging rabbit pregnancy, it was of interest to determine if 'Lipomul' itself would alter the physiological effect of endogenous progesterone.

New Zealand white rabbits, 21–26 days pregnant, were given 150–200 ml. 'Lipomul' intravenously during a period of 8–12 hr. This treatment resulted in premature labour. Rabbits pre-treated by 5 mgm. of progesterone, intramuscularly, could not be induced by higher doses of 'Lipomul'.

Increasing the amount of 'Lipomul' or the speed of infusion resulted in delivery with dead litter, while further increase led to maternal death in convulsions. Further experiments have shown that the active part of 'Lipomul' is the lecithin fraction. The other components of this emulsion[1] were not effective in producing premature labour.

The lecithin in 'Lipomul' is derived from soya bean phosphatides. It is still obscure whether or not pregnant mammals have phospholipids which could control the effect of progesterone. Work is in progress to purify further the soya bean phosphatides and to isolate from the blood and placenta of pregnant women a fraction with similar characteristics.

This work was supported by grants from the National Institutes of Health (*RG*-7942), the Population Council, and the Sigfrid Jusélius Foundation, to which we wish to express our thanks.

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A PERCHLORIC ACID–NAPHTHOQUINONE METHOD FOR THE HISTOCHEMICAL LOCALIZATION OF CHOLESTEROL

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THE histochemical demonstration of cholesterol by adaptations[1] of the Liebermann–Burchard reaction has never been satisfactory owing to diffusion of the coloured product and to destruction of the tissue by the concentrated sulphuric acid used in the method. The bismuth trichloride method[2] for cholesterol appears to be somewhat capricious when used in histochemistry[3]. The method to be described, a perchloric acid–naphthoquinone reaction, is more sensitive and gives better localization of cholesterol than either of the older methods.

Frozen sections from tissues fixed in calcium–formol are mounted on glass slides and dried in air. The reagent is 0·1 per cent 1,2-naphthoquinone-4-sulphonic acid in a mixture of ethanol–perchloric acid (60 per cent)–formaldehyde (40 per cent)–water (2 : 1 : 0·1 : 0·9 v/v). The sections are painted with reagent and heated on the bottom of an oven at 60–65° for 5–10 min. until the red colour which first appears turns dark blue. (Sections left floating in calcium–formol for some days react more briskly and intensely than those freshly cut.) A drop or two of 60 per cent perchloric acid is placed on the section and a coverslip lowered into position. The dark blue colour of cholesterol is stable in perchloric acid for a few hours and then gradually turns greyish black. The colour is not stable in either water or glycerine-jelly.

To test the specificity of the method, 5 mgm. samples of the following compounds were spot-tested on glass slides with the perchloric acid–naphthoquinone reagent.

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Lipids: Red turning dark blue: cholesterol (3-hydroxy, Δ^5), cholesterol oleate, cholesterol palmitate, cholesterol stearate, cholesterol chloroformate, 7-dehydrocholesterol (3-hydroxy, $\Delta^{5,7}$). Red turning pale blue: cholesterol acetate. Red: lanosterol (3-hydroxy, Δ^8). Black: α- and β-carotene mixture (15 : 85), crystalline vitamin A. Brown: oleic acid, linoleic acid, stearic acid, triolein, tristearin, ovolecithin, brain cephalin, brain cerebroside. No colour: cholestane, cholestanol (3-hydroxy), cholestanone (3-oxy), cholestenone (3-oxy, Δ^4), sphingomyelin.

Amino-acids: Pale pink turning yellow: tryptophan. Pink turning brown: tryptophan-formaldehyde carboline. No colour: tyrosine, phenylalanine, histidine, proline, hydroxyproline, arginine, cystine, cysteine, methionine, glycine.

Purines and Pyrimidines: No colour: adenine, guanine, xanthine, uracil, cytosine, thymine.

Carbohydrates: Pink turning brown: glucose, galactose, mannose, lactose, sucrose, inulin. No colour: inositol, glucosamine, galactosamine.

Other organic compounds: Dark blue: resorcinol. Yellow: phenol, catechol. Orange: indole. Brown to black tars: pyrrole, furfural. No colour: benzene, hydroquinone, pyridine, pyridoxine.

From these spot tests, it is concluded that 3-hydroxy-Δ^5 (or $\Delta^{5,7}$)-steroids and their esters are the only physiological substances which develop a dark blue colour with the perchloric acid–naphthoquinone reagent. Steroids without either the 3-hydroxyl or Δ^5 groups are unreactive, but a second double bond in ring *B* (for example, Δ^7) does not inhibit the

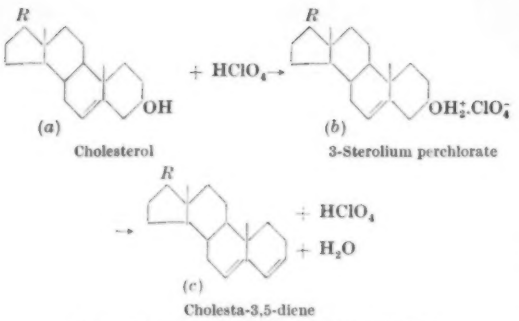

Fig. 1. The action of perchloric acid on cholesterol

reaction. The functional groups of the steroid nucleus demonstrated by this method are therefore essentially the same as those detected by the Liebermann–Burchard reaction[4].

The chemical mechanism of the perchloric acid–naphthoquinone reaction is unknown but previous investigations[5] have shown that perchloric acid has the specific effect, shared only with hexafluorophosphoric acid, of reacting with 3-hydroxy-Δ^5 steroids (Fig. 1*a*) to form insoluble 3-sterolium salts (Fig. 1*b*). The insolubility of these salts may explain the improved localization obtained with this method when compared with the Liebermann–Burchard reaction. By eliminating a molecule of water, excess perchloric acid produces cholesta-3,5-diene (Fig. 1*c*) from the sterolium salt, as does sulphuric acid from cholesterol[6]. Addition of acid to the 3,5-diene may be responsible for the transient red colour which is first formed, but the development of the dark blue colour appears to depend on a reaction between the diene and naphthoquinone. The diene structure is essential, since no colour develops in tissue sections brominated between the perchloric acid and naphthoquinone stages. Although naphthoquinones are dienophiles, Diels–Alder adduction of the diene to the quinone is not a plausible explanation for the reaction, for cholesta-3,5-diene does not undergo diene synthesis at the relatively low temperature used in the test[7]. A related method, using phenol and perchloric and molybdic acids, has been used to identify cholesterol on chromatograms[8]. A diene–quinone reaction may be the basis of both methods, for phenol is probably oxidized by these acids to a benzoquinone.

The specificity of the perchloric acid–naphthoquinone method for cholesterol was confirmed histochemically by the substantial reduction in colour after the tissue sections had been extracted in acetone at 4° for 18 hr. The success of extraction by acetone, in which cholesterol is highly soluble, indicates that the reaction in tissues is due to lipid. The slight residual staining after extraction may be due to the difficulty of removing all lipid from tissue sections[9] or to the binding of lipid to protein.

In rat tissues, a dark blue colour reaction is seen in the myelin sheaths of the central (Fig. 2) and peripheral (Fig. 3) nervous systems, in the zona fasciculata (Fig. 4) and glomerulosa of the adrenal cortex, in the interstitial cells of the testis and in the lutein cells of the corpus luteum. Slight staining is seen in neuroglial fibres but axons are unstained, as are the unmyelinated axons of the leg nerve of the lobster.

Elastic lamellæ in the normal human and rat aorta are unstained, even though these lamellæ contain lipid[10], including phospholipid[11]. Only traces of cholesterol are present in the interlamellar spaces of the media of the human and rat aorta (Fig. 5), but a strong reaction is given by the lipid-laden macrophages which are frequently found in the more superficial parts of the human arterial intima. The junctional zone between media and intima, the point where phospholipid first accumulates in the early lipoidal lesions of human atherosclerosis[12], contains relatively little cholesterol but the internal elastic lamella itself is sometimes faintly stained at this stage (Fig. 6). The lipid masses of the mature atherosclerotic plaque contain abundant cholesterol (Fig. 7).

A pale pink reaction is seen in the zymogen granules of the rat pancreas and submandibular gland and a weaker background colour of the same hue is seen in many tissues. It is concluded from the spot-tests that the pink colour in tissues is due to tryptophan and possibly carbohydrate, whereas the well-localized dark blue colour obtained with the perchloric acid–naphthoquinone method is a highly specific histochemical test for cholesterol and related steroids.

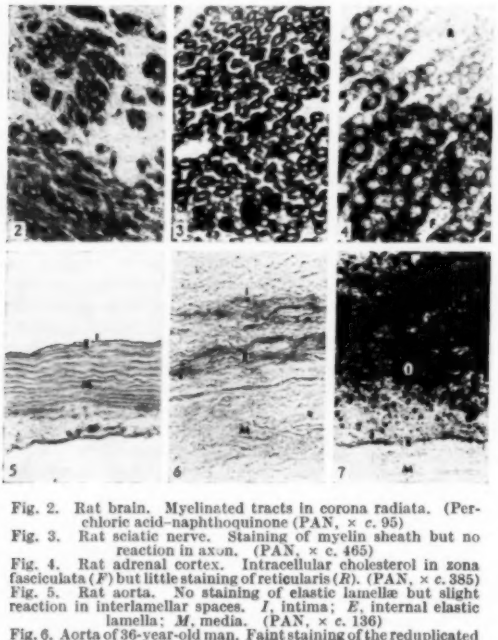

Fig. 2. Rat brain. Myelinated tracts in corona radiata. (Perchloric acid–naphthoquinone (PAN, × *c.* 95)

Fig. 3. Rat sciatic nerve. Staining of myelin sheath but no reaction in axon. (PAN, × c. 465)

Fig. 4. Rat adrenal cortex. Intracellular cholesterol in zona fasciculata (*F*) but little staining of reticularis (*R*). (PAN, × *c.* 385)

Fig. 5. Rat aorta. No staining of elastic lamellæ but slight reaction in interlamellar spaces. *I*, intima; *E*, internal elastic lamella; *M*, media. (PAN, × *c.* 136)

Fig. 6. Aorta of 36-year-old man. Faint staining of the reduplicated internal elastic lamella (*E*) and elastic fibres in the adjacent intima (*I*). (PAN, × *c.* 285)

Fig. 7. Aorta of 58-year-old man. Accumulation of cholesterol in intima (*I*) and around internal elastic lamella (*E*). (PAN, × *c.* 95)

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PROTEOLYSIS OF THYROGLOBULIN BY THYROID ENZYMES

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SINCE the thyroid hormone is held by peptide bonds within the molecule of its storage form, thyroglobulin, it is generally accepted that hydrolytic enzymes are concerned with its controlled release into the blood stream. The proteolytic activity of thyroid tissue has been shown to vary with the physiological status of the animal as, for example, that following the administration of thyrotrophin or of anti-thyroid drugs[1]. Thus, the purification and the study of the properties of the enzyme (or enzymes) concerned could afford more exact information as to the release mechanism and perhaps help to clarify some types of thyroid disease.

It has been demonstrated that iodinated tyrosines and thyronines can be liberated by incubation of thyroid homogenates[2], saline extracts of the gland[3] or by the action of a partially purified thyroid proteinase on thyroglobulin[4]. The enzyme preparation used by the last-named workers contained peptidase activity. Further purification studies[5] led to the separation of a peptidase fraction ('APATase'), which readily hydrolyses preferentially the pepsin substrate, acetyl-L-phenylalanyl-L-tyrosine[6] (APAT), and a proteinase of high specific activity. The proteinase still retained, however, some activity towards the peptide, L-cysteinyl-L-tyrosine, also a substrate for pepsin[7]. This property has been shown to be due to the presence of another peptidase ('cysteinyltyrosinase') which can be separated from the proteinase[8]. The actions of proteinase and APATase are not influenced by the usual enzyme activators, whereas cysteinyltyrosinase is metal-dependent[8].

The *p*H optimum of the proteinase is 3·5 (hæmoglobin substrate), while the two peptidases, APATase and cysteinyltyrosinase, show peaks of activity at *p*H 4·0 and 5·3 respectively. Like pituitary[9] and adrenal[10] acid proteinases, the thyroid enzyme, when freed from the two peptidases already mentioned, appears to have no action against simple peptide substrates.

We have carried out a series of experiments in which thyroid proteinase and APATase have been incubated, under varying conditions, with preparations of rat thyroglobulin labelled *in vivo* with iodine-131.

Thyroglobulin substrates were prepared as follows: eight female rats, average weight about 200 gm., were each injected intraperitoneally with 90–100 μc. carrier-free iodine-131 as iodide. 48 hr. later the animals were killed, the thyroid glands dissected out, pooled, blended by hand in a glass homogenizer with ice-cold isotonic saline (0·6 ml.), insoluble material removed by centrifugation and the supernatant brought to 42 per cent saturation with ammonium sulphate at 25° C. The precipitate, after standing for 2 hr., was separated, washed with 42 per cent ammonium sulphate, dissolved in water (0·6 ml.) and dialysed overnight. A precipitate which separated was discarded and the supernatant adjusted to 0·015 *M* *tris* buffer (*p*H 7·2) and 0·015 *M* ethylenediamine tetraacetic acid (EDTA), dialysed against the same concentrations of buffer and chelating agent (200 ml.) for 30 hr. and then against distilled water for 17 hr. The faintly opalescent salt-free solution was then heated in a sealed tube at 80° C. for 4 min. to reduce the content of endogenous proteinase, as it has been found that thyroglobulin tends to retain this enzyme during salt fractionation[11]. Protein was then precipitated by adding four volumes of acetone below − 5° C., followed by a small drop of saturated sodium chloride. The precipitate was dissolved in water (about 0·7 ml.), dialysed overnight and held in the frozen state until required.

The enzyme samples were prepared from pig thyroid glands, substantially as described[5], but were further processed by two treatments with buffered EDTA (as for thyroglobulin substrate), to reduce the activities of traces of metal-dependent peptidases, followed by prolonged dialysis against distilled water. The proteinase had an activity of 1,200 units/mgm. protein when incubated for 30 min. at 37° C. and *p*H 3·5 with bovine hæmoglobin substrate, followed by spectrophotometric determinations at 280 mμ (compare Anson[12]). This corresponds to a purification factor of about 400, a twenty-fold improvement on the preparation used in our previous work[4]. The peptidase (APATase) contained 8 units/mgm. protein, one unit being defined as that quantity of enzyme which liberates 1·0 μ*M* tyrosine from APAT (5 μ*M* per ml.) in 60 min. at 37° C. and *p*H 4·0. When incubated at a concentration of 1 mgm./ml. with APAT, the proteinase released the merest trace of tyrosine, as determined chromatographically, whereas APATase, under identical conditions but at a concentration of 0·1 mgm./ml., hydrolysed about 25 per cent of the substrate. The sample of APATase was slightly contaminated by proteinase (10 units/mgm. protein).

Action of purified proteinase on thyroglobulin. In our earlier investigations[4] with labelled thyroglobulin and partially purified proteinase, both prepared from pig thyroid, appreciable hydrolysis was observed at *p*H 3·5. However, in the present work, it became clear from the results of preliminary experiments, using rat thyroglobulin, that substantially greater quantities of labelled components were liberated at higher *p*H, even though the proteinase gave a smooth *p*H-activity curve with bovine hæmoglobin substrate, being only one-fifth as active at *p*H 5·2 as at *p*H 3·5.

In the following experiment the nature of the labelled substances released at the higher *p*H was examined. A half of a thyroglobulin solution prepared as above was mixed with an equal volume of 0·1 *M* ammonium acetate buffer *p*H 5·2. Duplicate tubes containing 0·1 ml. thyroglobulin solution and 0·05 ml. proteinase solution (equivalent to 60 units) were incubated for 2 and 16 hr., while water replaced the enzyme in duplicate control tubes for each period.

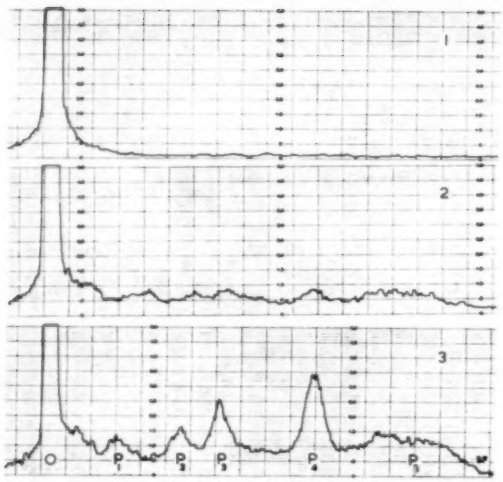

Fig. 1. Distribution of iodine-131 on paper chromatograms of thyroglobulin after incubation with thyroid proteinase at 37° C. and *p*H 5·2. Chromatograms developed with *n*-pentanol/propionic acid/water (20 : 3 : 15). (1) 16-hr. control; (2) after 2 hr.; and (3) after 16 hr. with thyroid proteinase. Scannings made with instrument set at: full-scale deflexion, 500 c.p.m.; slit width, ½ in.; scan speed, 3 in./hr.; time constant, 40 sec. *O* = origin; *SF* = solvent front; P_1–P_5 = main areas of radioactivity

A similar set, but buffered at *p*H 3·45 with 0·1 *M* ammonium acetate, was included. The tubes were tightly stoppered and shaken during incubation at 37° C. Following incubation, 0·025 ml. samples from each tube were spotted on to washed Whatman No. 3 paper and descending chromatograms developed with *n*-pentanol/propionic acid/water (PPW; 20 : 3 : 15 by vol.). Marker spots contained mono- and di-iodotyrosine and thyroxine (25, 25 and 50 μgm. respectively). Duplicate paper sheets (series *B*) were prepared with further 0·025 ml. samples from all tubes after these had been standing for 48 hr. in the frozen state. After cutting the sheets into strips the latter were scanned for radioactivity using a Nuclear Chicago 'Actigraph II' automatically recording instrument. Rapid scans indicated marked increases in the radioactive components released at *p*H 5·2 compared with *p*H 3·45. Accordingly, further examination was restricted to the incubations at the higher *p*H.

Fig. 1 illustrates the results of slow scanning of the strips corresponding to 2-hr. and 16-hr. tests and that of a 16-hr. control. Duplicates corresponded closely. Apart from the origin peak—presumably residual protein—five main areas of activity are discernible (P_1–P_5), each area increasing and becoming more clearly defined with the longer incubation period. The control scans showed only background activity except at the origin.

Approximate values for percentage hydrolysis were derived from estimates of the areas of migrated radioactivity and those at the origins. The results of the calculations are given in Table 1, which lists the duplicate values for the relevant strips from each of the two series of chromatograms (*A* and *B*) run in PPW with 0·025 ml. samples. On averaging each of the sets of four values, it appears that about three times as much radioactivity had been released after 16 hr. as after 2 hr. (approximately 27 and 10 per cent respectively). In view of the variations within the calculations, the exact agreement of three of the duplicate percentages must be regarded as fortuitous.

In order to identify the peaks of radioactivity, the residual solutions (0·1 ml.) from the incubation tubes were precipitated by addition, with warming, of ethanol to 70 per cent concentration, in the presence of carrier mono- and di-iodotyrosine, thyroxine and a sample of purified pig thyroglobulin (0·5 mgm.) prepared by the method of Derrien, Michel and Roche[13]. After centrifuging, the residues were washed with 0·2 ml. 70 per cent ethanol and the combined supernatants evaporated in micro beakers in a desiccator (phosphorus pentoxide). The residues were then spotted on to paper with ethanol/water/conc. ammonium hydroxide (7 : 2 : 1), the beakers being monitored for completeness of application, and chromatograms developed with PPW. The radioactivity scans were similar to those of the 0·025 ml. samples directly applied. The peaks corresponding to P_3 and P_4 (Fig. 1), which had the same mean R_F values (0·37; 0·59) as marker spots of mono- and di-iodotyrosine respectively, were further characterized by eluting the areas with the alkaline ethanol solvent and then developing chromatograms of the eluate residues in *n*-butanol / 2*N* acetic acid (1 : 1). Well-defined areas with peaks at R_F 0·43 and 0·54 accounted for nearly all the radioactivity on the individual strips. This was checked by radioautography and by subsequent spraying of the strips with ninhydrin to visualize the carrier iodo amino-acids, which demonstrated exact coincidence between radioactive and coloured zones. Although eluted and re-run in butanol/acetic acid the peaks P_1 and P_2 (as in Fig. 1) could not be characterized although P_2 did have a component at the same R_F as mono-iodotyrosine. It is possible that they represented, in part, small peptides. The elongated peak (P_5) of R_F 0·70–0·90 suggested a double character on radioautography, the higher part corresponding to marker thyroxine. On eluting and re-running in *n*-butanol/*n*-pentanol/2 *N* ammonium hydroxide (BPA; 1 : 1 : 2), radioactive peaks were found at R_F values 0·00, 0·08, 0·14 and 0·20, with a broad spread of radioactivity through R_F 0·32 corresponding to thyroxine. Thus, P_5 appeared to consist of a mixture, but the evidence strongly suggested thyroxine as one of the components. An unequivocal demonstration of the direct release of thyroxine by the proteinase preparation was obtained in the following experiment.

An apparent synergistic action of proteinase and APATase on thyroglobulin. In the preparation of the

Table 1. Hydrolysis of Thyroglobulin by Thyroid Proteinase at *p*H 5·2

Tests	Incubation period—2 hr.				Incubation period—16 hr.			
	A		*B*		*A*		*B*	
Migrated (*M*)	7,500*	7,060	10,800	11,000	26,000	27,800	24,000	29,200
Origin (*O*)	89,900	84,500	84,400	85,800	69,800	74,100	68,700	72,900
O + *M*	97,400	91,560	95,200	96,800	95,800	101,900	92,700	102,100
M as per cent *O* + *M*	7·7	7·7	11·4	11·4	27·2	27·3	25·9	28·6
Controls (origin)	92,400	93,900	99,100	98,200	101,700	106,400	97,500	99,500

* Figures are given as counts per min. of iodine-131 (corrected for decay back to day of injection).

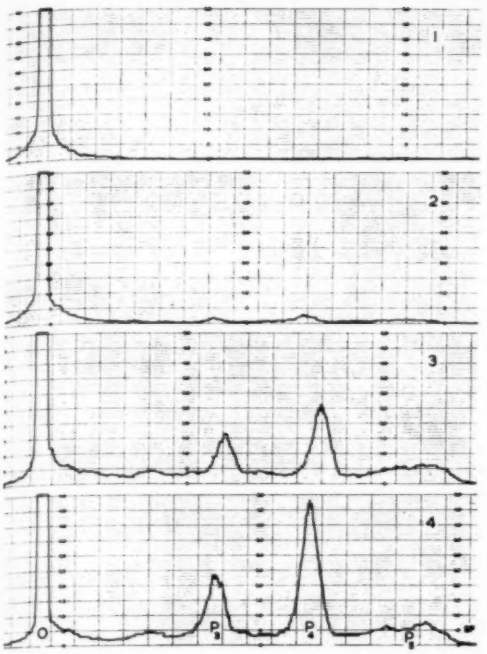

Fig. 2. Distribution of iodine-131 on paper chromatograms of thyroglobulin after incubation with thyroid proteinase and/or APATase at 37° C. and *p*H 5·25. Chromatograms developed with *n*-pentanol/propionic acid/water (20 : 3 : 15). (1) Control; (2) after 16 hr. with APATase; (3) after 16 hr. with proteinase; (4) after 16 hr. with proteinase plus APATase. Scannings made with instrument set at: full-scale deflexion, 1,500 c.p.m.; slit width, ½ in.; scan speed, 6 in./hr.; time constant, 20 sec. *O* = origin; *SF* = solvent front; P_3–P_5 = main areas of radioactivity

labelled thyroglobulin sample for this experiment the step involving dialysis against buffered EDTA was omitted as the chelating agent was present during incubation. The thyroglobulin solution was adjusted to 0·015 *M* ammonium acetate, *p*H 5·25, and 0·015 *M* EDTA, and 0·1 ml. added to each of eight incubation tubes which were tightly stoppered and pre-incubated for 1 hr. at 37° C. Duplicate tubes then received 0·05 ml. of solutions of (*a*) proteinase (60 units), (*b*) APATase (0·4 unit) and (*c*) proteinase (60 units) plus APATase (0·4 unit), the two control tubes having water only added. After 16 hr. incubation 0·025 ml. samples were applied to paper, run in PPW and the strips scanned as in the previous experiment. The tracings are shown in Fig. 2. Duplicates agreed closely. In the case of the proteinase alone there are again well-marked peaks (P_3, P_4) corresponding to mono- and di-iodotyrosine. Noteworthy is the virtual absence of peaks P_1 and P_2 (compare Fig. 1) and the greater relative concentration of radioactivity in the higher R_F region of P_5 coincident with thyroxine marker. Most striking, however, is the considerable augmentation of radioactivity in the areas corresponding to mono-iodotyrosine and, particularly, di-iodotyrosine, in the tracings of the samples where the two enzymes were associated. APATase alone released very little radioactivity and this could have been due to a trace amount of proteinase in the APATase sample (equivalent to 0·5 proteinase unit in the 0·15 ml. of incubated solution). The contributions of the three main areas, P_3, P_4 and P_5, to the migrated radioactivity, in the case of the proteinase alone and proteinase plus peptidase, as derived from the 0·025 ml. scans, are shown in Table 2.

Table 2. Contributions of Radioactive Areas, P_3, P_4 and P_5 (Fig. 2), to Total Migrated Radioactivity following Treatment of Thyroglobulin with (*a*) Proteinase alone and (*b*) Proteinase plus APATase at *p*H 5·25

Radioactive area	Proteinase (duplicates)		Proteinase + APATase (duplicates)	
P_3	4,900*	5,200	7,400	8,300
P_4	7,900	7,900	15,100	15,700
P_5	6,700	6,600	7,900	8,400
Total migrated	28,100	28,700	39,300	40,900
Total migrated less P_3, P_4, P_5	8,600	9,000	8,900	8,500

* Figures are given as counts per min. of iodine-131 (corrected for decay back to day of injection).
P_3 = mono-iodotyrosine; P_4 = di-iodotyrosine; P_5 = thyroxine-containing area.

The high R_F radioactive areas (P_5) for both proteinase and combined enzymes were unequivocally demonstrated to contain thyroxine as one of the components by further chromatographic examination of the residual solutions (0·1 ml.) from the relevant incubation tubes. Following development in PPW and elution of the areas coincident with marker thyroxine, the duplicate eluates were combined, carrier thyroxine added and the eluate residues then rechromatographed with the solvent system BPA. The 'thyroxine' areas were eluted and the residues re-run in *n*-butanol / 2*N* acetic acid (1 : 1). The chromatograms were checked by radioautography at each stage. Fig. 3 depicts the final tracings, which show in each case a well-defined thyroxine area at R_F 0·72 which coincided exactly with the carrier thyroxine—visualized by ninhydrin—with a minor peak of higher R_F which was not characterized. The total radioactivity in the thyroxine areas was higher when the enzymes were associated but, in view of the involved chromatographic and elution procedure, this difference may not be significant.

It may be concluded from the above results that thyroid acid proteinase is able to degrade thyroglobulin with the release of mono- and di-iodotyrosine and thyroxine. However, it is also possible that even the trace amount of APATase in the purified proteinase sample was the catalyst responsible for the release of the iodo amino-acids from peptides

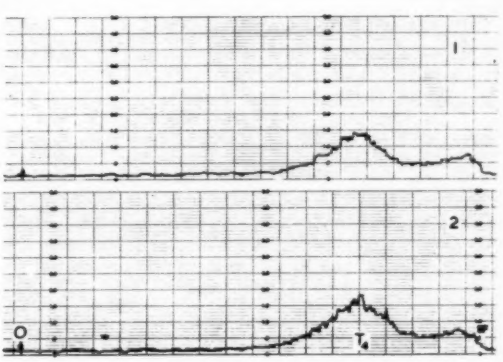

Fig. 3. Demonstration of thyroxine (T_4) on final paper chromatograms of eluates of P_5 (see Fig. 2). Chromatograms developed with *n*-butanol / 2*N* acetic acid (1 : 1). (1) Corresponding to incubation with proteinase; (2) corresponding to incubation with proteinase plus APATase. Scannings made with instrument set at: full-scale deflexion, 500 c.p.m.; slit width, ½ in.; scan speed, 3 in./hr.; time constant, 40 sec.
O = origin; *SF* = solvent front; T_4 = thyroxine

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produced by the proteinase as intermediates. In any event, as the peptidase had almost negligible action on intact thyroglobulin, it seems clear from the results that the two enzymes can act synergistically in the hydrolysis of the protein. It is not possible at this stage to make a concise evaluation of the respective contributions of the two enzymes, particularly as specific activities were not determined. In this connexion our earlier observation[4] may be recalled that the specific activities of enzymically released mono- and di-iodotyrosine were higher than those of the amino-acids isolated from the same sample of labelled thyroglobulin after complete chemical hydrolysis. This implied, possibly, a 'terminal turnover' in the biosynthesis and degradation of the iodo amino-acids.

Our present investigations are designed to clarify further the nature of the primary actions of thyroid acid proteinase and peptidases.

Mr. R. E. Loughlin was associated in preliminary experiments of this investigation. One of us (J. D. M.) has held the Thomas and Elizabeth Ross Scholarship and we are also indebted to the National Health and Medical Research Council for a grant in aid of this work.

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DEMONSTRATION OF INTERACTION PRODUCTS OF ADENINE AND ADENOSINE PHOSPHATES WITH FORMALDEHYDE

By Dr. D. L. WOODHOUSE

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RECENTLY, Alderson[1] ascribed a special role to adenylic acid as the mediator of the mutagenic action of formaldehyde on *Drosophila* larvæ, the main postulated agent being an 'adenylate dimer' produced by the interaction of formaldehyde with the amino-group of this purine derivative in the food mixture. The evidence for such interaction products rests mainly on slight alterations in the ultra-violet absorption spectra[2] after incubation of purine bases, nucleotides, etc., with formaldehyde. In view of the scheme suggested by Alderson[1] for the utilization of the postulated metabolite in deoxyribonucleic acid synthesis and its role in mutagenic activity, further demonstration and, if possible, isolation of such reaction products seemed desirable: experiments have been made therefore to see whether they could be resolved from the reaction mixture by chromatography or paper electrophoresis.

In preliminary tests adenine, 2 mgm./ml., was incubated for 18 hr. at 38° C. in presence of 0·1 per cent formaldehyde. After confirming the effect on the maximum of the absorption curve, 20 μgm. samples (10 μl.) were chromatographed by the ascending method using various solvent systems. The positions of the areas containing purine were made visible by the usual method of photographing with ultra-violet light. Using isopropanol / 2*N* hydrochloric acid (65 : 35) evidence of several compounds was obtained, some with greater R_F values and some with less R_F values than adenine (Fig. 1). This is in accordance with the possibility of the formation of monomethylol and dimethylol derivatives, and the 'dimer' (A—CH_2—A) containing the methylene bridge by conjugation of two adenine residues. Formaldehyde may also react with the imino group at the 9 position of the free purine ring. Adenosine, after reaction with formaldehyde, gave evidence of a component moving faster than the adenosine comparison spot in the isopropanol–hydrochloric acid solvent and in methanol / 90 per cent formic acid / water (160 : 30 : 10).

When adenosine-3′-phosphate was examined after formaldehyde treatment, no separation of components was obtained. In all cases clean separation of the products, some of which are relatively unstable, seemed to be disturbed by the long exposure to the solvents, as shown by trailing.

Experiments were made therefore to see whether useful separation could be obtained by treatment with various ion-exchange materials including types of synthetic resins. When solutions of adenosine and formaldehyde-treated adenosine containing 41 μgm./ml. were exposed for 2 hr. to 'Amberlite *IRC* 50 (*H*)' resin, 0·5 gm.–8 ml., a much higher proportion of the unchanged adenosine was abstracted as shown by the absorption curves in Fig. 2, and by Table 1. Complete separation of the interaction products from the mixture was not achieved, however.

By paper electrophoresis a clear demonstration of reaction products was obtained with formaldehyde.

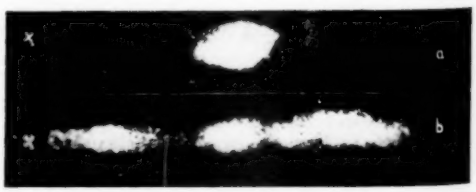

Fig. 1. Chromatography of adenine: (*a*), before; (*b*), after treatment with formaldehyde

Table 1. Effect of Ion-exchange Resin on Adenosine and Formaldehyde-treated Adenosine

mμ	Optical density before resin treatment*		Optical density after resin treatment	
	Adenosine	Adenosine treated with formaldehyde	Adenosine	Adenosine treated with formaldehyde
250	0·85	0·78	0·398	0·695
252	—	—	0·445	0·80
254	1·008	0·95	0·482	0·90
256	—	—	0·51	0·98
258	1·125	1·18	0·53	1·075
260	1·156	1·24	0·545	1·15
262	1·15	1·28	0·545	1·22
264	1·131	1·31	0·538	1·26
266	1·084	1·33	0·517	1·27
268	1·019	1·31	0·483	1·27
270	0·92	1·23	0·438	1·22

* Diluted 1–200 for analysis.

treated adenine (20 μgm.) or adenosine-3′-phosphate (60 μgm.); 2 m.amp. per 5 cm. strip for 5 hr. in 0·2 *M* acetate–acetic acid buffer, *p*H 4. Adenine–formaldehyde compounds with less mobility than the untreated marker were detected (Fig. 3) while adenosine-3′-phosphate showed a well-separated spot with greater mobility than the unchanged compound. Formaldehyde-treated adenosine solutions did not give good separation. It would appear that the structures of the riboside and any reaction products are too similar to be influenced differentially by the 'transporting charge' but that the phosphate group confers properties allowing the relatively small effect of the formaldehyde group to be utilized in the separation. Resolution was further improved when 0·1 *M* buffer (4 m.amp. per strip) was used for 3–4 hr. (Fig. 4). Similar separation of products from adenosine-5′-phosphate after exposure to formaldehyde is illustrated in Fig. 5, which shows the unchanged ribotide, and one (possibly two) compounds with greater mobilities than the 5′-phosphate.

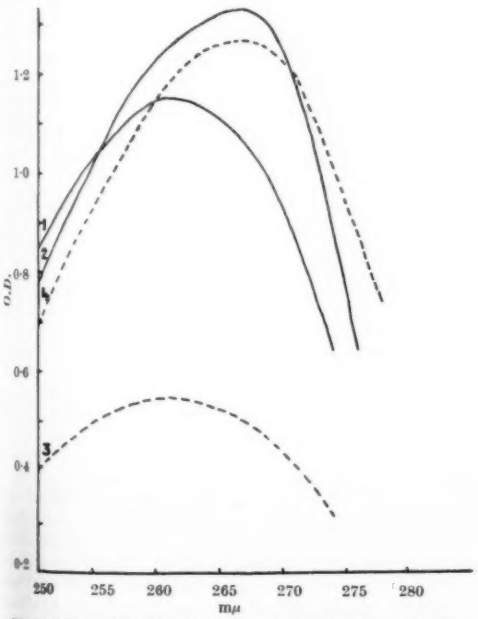

Fig. 2. Differential effect of resin on adenosine and formaldehyde-treated adenosine; ultra-violet absorption curves: 1, adenosine; 4 mgm./ml. (diluted 1–200 for analyses); 2, adenosine after exposure to formaldehyde: 4 mgm./ml. (diluted 1–200 for analyses); 3, solution as in 1, after treatment with resin '*IRC* 50 (*H*)' (undiluted); 4, solution as in 2, after treatment with resin (undiluted)

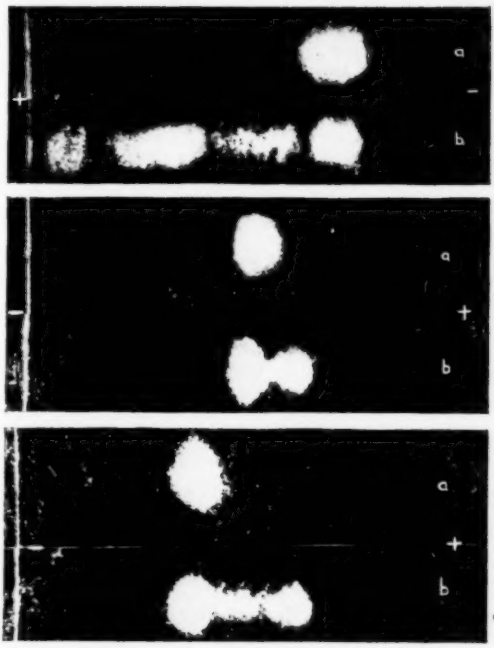

Fig. 3 (top). Electrophoresis of adenine (*a*) before; (*b*), after treatment with formaldehyde. 0·1 *M* acetate buffer, *p*H 4·0, 4 m.amp. per 5 cm. strip, 3 hr.

Fig. 4 (centre). Electrophoresis of adenosine-3′-phosphate (*a*) before; (*b*), after treatment with formaldehyde. Conditions as for Fig. 3

Fig. 5 (bottom). Electrophoresis of adenosine-5′-phosphate (*a*) before; (*b*), after treatment with formaldehyde. Conditions as for Fig. 3

The resolution of the nucleotides allowed a computation of the proportions of the components to be made. First, from the dried papers the areas denoted by the positions of the spots on the photographs were cut out, extracted with 4 ml. 0·1 *N* sulphuric acid and the content estimated by ultra-violet spectrophotometry.

From the figures for adenosine 3′-phosphate electrophoresis strips given in Table 2 it can be calculated that some 77 per cent of the nucleotide deposited on the paper was present as unchanged ribotide. Since it was evident from the absorption curves of the

Table 2. Optical Density of Solutions of Separated Components of Formaldehyde-treated Adenosine-3′-Phosphate

mμ	Control adenosine phosphate spot	Formaldehyde-treated Companion adenosine phosphate spot	Subsidiary-component spot
252	0·497	0·398	0·115
254	0·529	0·408	0·122
256	0·558	0·433	0·127
258	0·543	0·418	0·125
260	0·518	0·398	0·119
262	0·484	0·372	0·114

Extracted after drying paper at 110° C. with 4 ml. 0·1 *N* sulphuric acid, using extract of blank paper for comparison

Table 3. Optical Density of Solutions of Separated Components of Formaldehyde-treated Adenosine-3′-Phosphate

mμ	Formaldehyde-treated Main spot	Subsidiary spot
254	0·288	0·128
256	0·31	0·137
258	0·313	0·143
260	0·310	0·150
262	0·30	0·153
264	0·283	0·152
266	0·26	0·144

Extracted from undried paper with 4 ml. 0·1 *N* sulphuric acid.

relevant solutions that during drying the formaldehyde combination was reversed to a considerable extent, extraction from an undried paper was also carried out. In this case it is difficult to be sure that the positions for the separated components are selected accurately since they must be judged by the photograph of a dried paper run in parallel. However, the substance which had a greater mobility than the original ribotide then gave an absorption maximum at 363 mμ compared with 258 mμ for adenylic acid under the same conditions (Table 3).

This work was carried out with the support of the Birmingham Branch of the British Empire Cancer Campaign.

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GLUTAMYL PEPTIDASES IN RAT AND GUINEA PIG KIDNEY SLICES

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Histochemical Demonstration of γ-Glutamyl Transpeptidase-like Activity

UNTIL now, only the purely hydrolytic character of the amidase group of enzymes has been demonstrated histochemically. Recent reports[1,2] have described methods for the colorimetric estimation in serum and tissues of enzyme-catalysed cleavage of γ-L-glutamyl peptides possibly mediated by γ-glutamyl transpeptidase[3]. In order to localize this enzymic activity to tissue sections at a histological level a potentially chromogenic substrate *N*-(γ-L-glutamyl)-β-naphthylamide was synthesized for use in a simultaneous azo-coupling reaction.

The synthesis of *N*-(α-benzyl-N^{α}-carbobenzoxy-γ-L-glutamyl)β-naphthylamide was performed from N^{α}-carbobenzoxy-1-glutamyl α-benzyl ester[4] and β-naphthylamine using the mixed anhydride technique with isobutyl chlorocarbonate in dioxane. The product was crystallized from ethyl acetate–petroleum ether, and had a melting point of 142–44° C. *N*-(γ-L-glutamyl)β-naphthylamide was obtained by catalytic hydrogenation in the presence of palladium black in 80 per cent acetic acid as solvent. The product was crystallized from dilute acetic acid; its melting point was 228° C. (Analysis found: C, 66·4; H, 5·9; N, 10·2; carboxyl-N, 4·9 (ref. 5). Calc. for $C_{15}H_{16}O_3N_2$: C, 66·2; H, 5·9; N, 10·3; carboxyl-N, 5·1). $[\alpha]_D^{20} + 2{\cdot}0°$ (*c*, 1·2 in dimethylformamide made 2 *N* with concentrated hydrochloric acid).

Kinetics of the release of naphthylamine from *N*-(γ-L-glutamyl)β-naphthylamide in a biochemical system using previously reported methods were investigated with rat kidney homogenates. The substrate, which was dissolved in 0·1 *N* sodium hydroxide and then neutralized, was used at a concentration of 0·001 *M* in 0·1 *M* *tris*(hydroxyamino)methane buffer at *p*H 8·5 with 0·0164 mgm. wet weight kidney/ml. No spontaneous hydrolysis of the substrate was noted. Pronounced activation by 0·003 *M* glycylglycine was noted with inhibition by γ-L-glutamyl-L-alanine but no inhibition by α-L-glutamyl-L-valine. There was complete inhibition by disodium phenoltetrabromophthalein sulphonate ('Bromsulphalein')[1]. It was of interest that release of naphthylamine from the naphthylamide substrate was enzymically catalysed many times faster than release of aniline from an analogous DL-anilide compound previously reported[1].

Histochemical work on the characteristics of enzymic release of naphthylamine from *N*-(γ-L-glutamyl)β-naphthylamide was performed predominantly on slide-mounted freshly frozen 10μ sections of guinea pig kidney using an incubating solution consisting of 0·5 ml. of a 4 mgm./ml. (0·013 *M*) stock suspension of substrate in acetone, 0·5 ml. of a 20 mgm./ml. (0·150 *M*) aqueous solution of glycylglycine in 10 ml. of 0·1 *M* *p*H 7·2 *tris* buffer, 28 ml. of water and 1 ml. of a solution containing 15 mgm. of the stable diazonium salt of *o*-aminoazotoluene (fast garnet *GBC*) at 37° C. for 20 min. A sub-optimal *p*H for transpeptidation[3] was used to facilitate simultaneous azo-coupling of the naphthylamine enzymically released. No effect on enzymic hydrolysis was noted after the addition to the incubating medium of 0·01 *M* L-glutamine, 0·01 *M* α-L-glutamyl-L-asparagine, 0·001 *M* magnesium chloride, 0·005 *M* *p*-chloromecuribenzoate, 0·001 *M* sodium iodoacetate, 0·01 *M* and 0·0005 *M* potassium cyanide, 0·01 *M* disodium ethylenediamine tetraacetic acid, 0·001 *M* sodium pyruvate, 0·0001 *M* di-iso-propylphosphorofluoridate or 0·004 *M* sodium taurocholate. 'Bromsulphalein' (0·0001 *M*) completely inhibited enzymic activity as did 0·005 *M* oxidized glutathione. No evidence of enzyme activation was noted using 0·01 *M* phosphate buffer at *p*H 7·2. When acetone-fixed paraffin embedded or acetone-washed frozen sections were incubated in the presence of glycylglycine as the γ-glutamyl acceptor, an estimated 50 per cent increase of activity was noted over incubation in its absence.

From the above histochemical and biochemical evidence it can be concluded that the enzyme which releases naphthylamine from *N*-(γ-L-glutamyl)-β-naphthylamide cannot be identified with glutaminase I since there was no inhibition by either *p*-chloromercuribenzoate or L-glutamine and phosphate did not activate the system. Moreover, it is unlikely that a sulphydryl-dependent enzyme, such as glutaminase I, would be demonstrated by a simultaneous azo-coupling reaction since the sulphydryl group would either be oxidized by, or coupled to, the diazonium reagent. Aminopeptidases described histochemically do not appear to be implicated since potassium cyanide failed to affect the reaction. In view of the above findings and the fact that enzymic release of naphthylamine is inhibited by oxidized glutathione and 'Bromsulphalein' and activated by glycylglycine[1], it would appear that the enzyme demonstrated has many of the characteristics of γ-glutamyl transpeptidase as defined biochemically[1,3].

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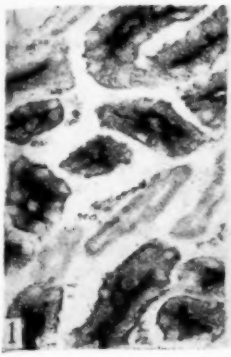

Fig. 1. Enzymic activity in brush border of proximal convoluted tubule

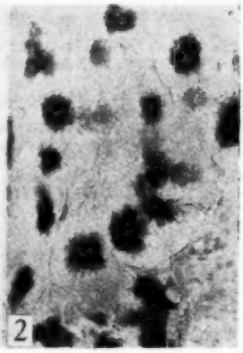

Fig. 2. Enzymic activity in acinar cells of pancreas. No activity evident in islet on the right

With freshly frozen tissues, acetone-washed or acetone-fixed and freeze-substituted paraffin-embedded tissues, γ-glutamyl transpeptidase-like activity was localized in the guinea pig kidney to the brush border of the proximal tubules in the outer medulla and the medullary rays (Fig. 1), and in the pancreas in acinar cells with no reaction evident in islets (Fig. 2). Although transferase reactions have been demonstrated histochemically previously by localizing the synthesized product of the reaction, this report is the first published description of a histochemically demonstrated transferase reaction in which kinetics of the reaction could be investigated during incubation.

Histochemical Characterization of a Unique α-Glutamyl Peptidase Activity

The use of histochemical substrates as a means of localizing known enzymic activities to specific cells or groups of cells in tissue sections has been well established. Histochemical techniques have also been used to distinguish selectively previously undescribed enzymic activity in single cells or groups of cells. The purpose of this communication is to report the identification and characterization of an enzyme(s) which catalyses the hydrolysis of *N*-(α-L-glutamyl)β-naphthylamide in the β-cells of the islets of Langerhans and the interlobular duct epithelium of the guinea pig pancreas.

The intermediate, *N*-(γ-benzyl-*N*$^{\alpha}$-carbobenzoxy-α-L-glutamyl)β-naphthylamide, was prepared from *N*-carbobenzoxy-L-glutamyl-γ-benzyl ester[6] and β-naphthylamine using the mixed anhydride technique with isobutyl chlorocarbonate in dioxane. The product was crystallized from ethyl acetate, and had a melting point of 170° C. The substrate, *N*-(α-L-glutamyl)β-naphthylamide, was obtained by catalytic hydrogenation of the carbobenzoxybenzyl ester intermediate in absolute methanol. The substrate was crystallized from 90 per cent methanol-ether and had a melting point of 174–75° C. (Analysis found: C, 62·3; H, 6·2; N, 9·3; weight loss on drying at 100° C. under vacuum, 5·8; amino-N, 4·9 (ref. 7); Calc. for $C_{15}H_{16}O_3N_2.H_2O$; C, 62·1; H, 6·2; N, 9·7; weight loss, 6·2; amino-N, 4·8 $[\alpha]_D^{20}$ + 42·5° (*c*, 1·0 in dimethylformamide made 2 *N* with concentrated hydrochloric acid).

Initial investigations using a simultaneous coupling technique revealed localization of enzymic activity in the guinea pig pancreas to the β-cells of the islets of Langerhans (Fig. 3) and the epithelium of the interlobular ducts (Fig. 4). The *p*H optimum with *tris*(hydroxyamino)methane buffer was 6·2. Histochemical work was performed on slide-mounted fresh-frozen 10μ sections of guinea pig pancreas employing an incubating solution containing 0·105 mgm. of substrate and 0·375 mgm. of the stable diazonium salt of *o*-aminoazotoluene (fast garnet salt *GBC*) per ml. of 0·2 *M tris* buffer, *p*H 6·2 for 30 min. at 27° C. Substrate was added as a stock solution (4·2 mgm./ml.) in acetone in order to facilitate its dissolution.

Complete inhibition of enzymic activity was obtained with 0·01 *M* disodium ethylenediamine tetraacetic acid, 0·01 *M* citrate, 0·01 *M* phosphate or 0·001 *M* cadmium chloride, while marked activation of hydrolysis was evident on the addition of 0·001 *M* calcium chloride. The addition of 0·001 *M* potassium cyanide or 0·001 *M* cobaltous chloride resulted in a moderate activation. No significant effect on enzymic activity was evident with 0·001 *M* magnesium or manganous chloride nor with 0·001 *M* iodoacetate, 0·005 *M* *p*-chloromercuribenzoate, 0·001 *M* zinc sulphate, 0·0001 *M* di-iso-propylphosphorofluoridate or 0·001 *M* nickel chloride. The peptide α-L-glutamyl-L-phenylalanine caused pronounced inhibition of activity at 0·01 *M* concentration while no inhibition was observed with the same concentration of L-isoglutamine, L-asparagine, L-leucinamide, benzoyl-DL-arginine amide, L-glutamine, L-glutamic acid or glycylglycine.

From the above histochemical evidence it would appear that the enzyme(s) responsible for the hydrolysis of *N*-(α-L-glutamyl)β-naphthylamide in the β-cells of the islets of Langerhans and the interlobular duct epithelium of the guinea pig pancreas is activated by calcium, and inhibited by disodium ethylenediamine tetraacetic acid; in other words, it is a metal-activated enzyme. The fact that activity is not inhibited by leucinamide and the lack of effect of magnesium on the hydrolysis-rate of the histochemical substrate would tend to exclude the significant participation of the enzyme leucine aminopeptidase[8] in a hydrolytic reaction in the above sites. No other histochemical peptidase substrates including an *N*-(γ-L-glutamyl)β-naphthylamide (see previous communication) have been reported to be hydrolysed by an enzyme(s) having the activity characteristics or localizations of that reported here.

Many new potential peptidase substrates have been synthesized in attempts to characterize further this enzymic activity. Among these are the β-L-aspartyl,

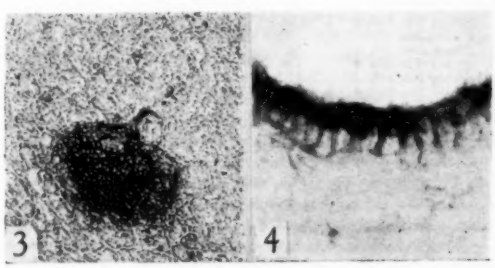

Fig. 3. Enzymic activity in β-cells of guinea pig pancreatic islet.

Fig. 4. Enzymic activity in epithelium of interlobular pancreatic duct

α-L-aspartyl, α-L-asparaginyl and α-L-glutaminyl-β-naphthylamides. None of the foregoing compounds was hydrolysed enzymically in the duct epithelium or β-cells of the islets of Langerhans of the guinea pig pancreas.

Attempts to purify the enzyme(s) responsible for the hydrolysis of *N*-(α-L-glutamyl)β-naphthylamide are in progress and a more detailed report of the kinetics and tissue localization of this activity will be the subject of a future communication.

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ANTIBIOSIS AMONG LACTOBACILLI

By H. C. DE KLERK and Prof. J. N. COETZEE

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In an investigation of lysogenicity in lactobacilli, 199 strains of lactobacilli were examined. One hundred and eighty-nine of these strains were isolated from as many different sources of human saliva, and many have previously been used in attempts to isolate lytic phage[1]. They comprised 140 homofermentative and 49 heterofermentative species. The remaining ten strains are type-cultures kindly supplied by Dr. M. Elizabeth Sharpe, of the National Institute for Research in Dairying, Reading, England. The composition of the tomato dextrose broth and agar used has been mentioned in ref. 1 and all cultures were incubated at 37° C. in an atmosphere of carbon dioxide. Lysogenicity was investigated as follows: supernatants of 10-day-old broth cultures were sterilized by shaking with a 1 per cent volume of chloroform. After all traces of chloroform had been evaporated, drops of these supernatants were spotted on lawns of all remaining strains. Plates were then incubated overnight. The supernatants of twelve cultures produced clear areas of inhibition of growth. On occasions a few colonies composed of resistant variants of the indicator strain were present in the zone of clearing. Sub-cultures of pieces of agar cut from the clear areas never showed growth. The maximum dilution in which supernatants are active is 1 : 10. Afterwards these twelve supernatants were tested against a further 269 homofermentative and 86 heterofermentative salivary strains of lactobacilli. Three of the supernatants, Nos. 253 *A*, 713 *A* and 838 *A*, possess identical spectra of activity. The remainder show very little overlapping and a total of 127 strains of lactobacilli are killed by the twelve supernatants. Results are shown in Table 1. Cultures from which active supernatants are derived are also inhibitory as indicated in Fig. 1.

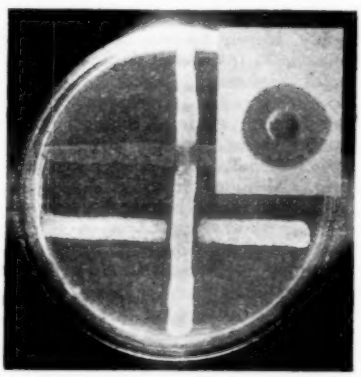

Fig. 1. Fermenti strain 466 washed and streaked on agar. Strain *F* 1 (Reading fermenti) and fermenti 206 cross-streaked. Plates incubated anaerobically overnight. Strain *F* 1 inhibited by strain 466

Inset: 0·2 ml. of a resuspended ammonium sulphate precipitate (*p*H 6·8) of supernatant 466 deposited in sealed hole in agar. After 8 hr. surface covered with sloppy agar containing strain *F* 1 and incubated. Area of inhibition of growth due to diffusion of antibiotic

Table 1

Supernatants producing zones of inhibition	Number of susceptible strains*				
	A (215)	*B* (1)	*S* (43)	*H* (1)	*F* (121)
Strain No. 35 *A*	2	1	1	1	1
Strain No. 93 *A*	8	1	1	1	15
Strain No. 124 *A*	0	0	1	0	1
Strain No. 185 *A*	6	1	1	1	9
Strain No. 253 *A*	15	1	1	1	20
Strain No. 303 *A*	14	1	1	1	19
Strain No. 496 *A*	11	1	1	1	17
Strain No. 558 *A*	20	1	1	1	21
Strain No. 571 *A*	3	1	0	1	3
Strain No. 713 *A*	15	1	1	1	20
Strain No. 838 *A*	15	1	1	1	20
Strain No. 466 *F*	2	0	0	0	7
Total number of different susceptible strains	51	1	1	1	73

* No activity demonstrated on 49 strains of *Lactobacillus casei*, 95 strains of *L. casei* var. *rhamnosus*, 13 strains of *L. plantarum* and 16 strains of *L. brevis*.

Figures in brackets indicate number of strains tested.

A, *L. acidophibus*; *B*, *L. bulgaricus* (Reading strain); *S*, *L. salivarius*; *H*, *L. helveticus* (Reading strain); *F*, *L. fermenti*.

The properties of active supernatants differ from those of phage suspensions in that no individual plaques are ever produced, activity is diffusable in agar (Fig. 1) and bactericidal titres are not affected by boiling for 15 min. The *p*H of the supernatants varies between 3·6 and 4·1, but activity is not affected by adjusting to *p*H 7 and/or conducting the tests on agar buffered to *p*H 7 with 1 per cent phosphates. In subsequent work the *p*H of supernatants was adjusted to 6·8. Microscopical observation of sensitive cultures treated with active supernatants demonstrated that the bactericidal action is not due to bacteriolysis and may correspond to the mode of

action of colicins. Particular attention was paid to avoiding the pitfall of mistaking this inhibition of catalase-negative organisms for the mere action of hydrogen peroxide which may have accumulated in the supernatants. Using the methods of Coulthard *et al.*[2], the hydrogen peroxide sensitivity of various strains was determined. In no case is an inhibitory strain more resistant to hydrogen peroxide than its indicator lactobacilli and it is sometimes more sensitive. Activity is not diminished by pre-incubating the supernatants with 0·1 per cent catalase (British Drug Houses) or using indicator strains seeded on agar supplemented with 10 per cent fresh rabbit serum. Furthermore, it is possible to increase the titre of supernatants considerably by dialysis against 30 per cent polyethylene glycol. Activity is not extractable with ether but is precipitated by saturated ammonium sulphate and also by ethanol. The antibiotics often attain maximal bactericidal titres in the supernatants of overnight cultures. Titres may decline on further incubation, and the detection of twelve antibiotic-producing cultures with the use of 10-day-old supernatants must be regarded as a minimal finding. The antibacterial spectrum of the twelve supernatants (concentrated by ammonium sulphate precipitation) was tested on other members of the family Lactobacteriaceae, various enterobacteria and staphylococci. A few strains of *D. pneumoniae* and many isolates of enterococci are susceptible to a number of these antibiotics. Enterobacteria and staphylococci appear to be immune. The action of these antibiotics thus appears restricted to certain members of the family Lactobacteriaceae in much the same way as colicine activity is confined to the family Enterobacteriaceae.

Apart from the spectrum of activity, no other differences have been noted between antibiotics derived from the eleven homofermentative strains and the single heterofermentative species. Reactions to these antibiotics may serve as a useful complement to phage-typing schemes of lactobacilli[1].

Vincent *et al.*[3] have considered the claims of workers who reported antibiotic effects produced by various homofermentative lactobacilli. They conclude that the hydrogen peroxide effect can only be excluded in the report of Grossowics *et al.*[4] and in their own particular case. The antibiotic reported by Grossowics *et al.* is produced by an unidentified homofermentative lactobacillus and is soluble in ether. It is active on a large series of organisms but no activity against lactobacilli is indicated. The antibiotic described by Vincent *et al.* is produced by strains of *L. acidophilus*. It is only produced by aged agar cultures, is inactivated by ethanol and possesses a wide spectrum, including activity on certain *L. acidophilus* strains.

The antibiotics described here differ in many respects from the two just mentioned. How their activity influences the ecology of natural populations of lactobacilli remains to be determined.

This work is submitted with the permission of Prof. C. L. de Jager, honorary director of the Nutrition and Dental Health Research Group of the South African Council for Scientific and Industrial Research.

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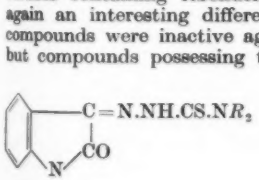
AGENTS WITH HIGH ACTIVITY AGAINST TYPE 2 POLIOVIRUS

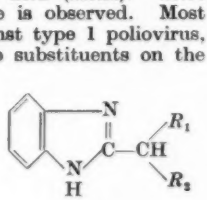
By Dr. D. G. O'SULLIVAN and Dr. P. W. SADLER

Middlesex Hospital Medical School, London, W.1

ISATIN-β-thiosemicarbazone (I ; $R_1 = R_2 = H$) and some derivatives in which the terminal amino-group remains unsubstituted (that is, I ; $R_2 = H$) have high selective activity against neurovaccinia[1], alastrim[2], rabbitpox (in tissue culture)[3] and smallpox[4], but show no inhibiting propensities towards ectromelia[4]. When, however, both hydrogen atoms at the end of the side-chain have been replaced by alkyl groups (I ; R_2 = alkyl), marked activity against ectromelia is introduced, but the compounds now have no inhibiting action against vaccinia[5]. Following these observations on the effect of isatin derivatives on pox viruses containing deoxyribonucleic acid (DNA), assays have been carried out using enteroviruses containing ribonucleic acid (RNA). Here again an interesting difference is observed. Most compounds were inactive against type 1 poliovirus, but compounds possessing two substituents on the terminal amino-group (I ; R_2 = alkyl) exhibited high activity against type 2 poliovirus.



(I)



(II)

Tamm and his co-workers[6] have recently studied and surveyed activities of promising compounds (benzimidazoles and benzotriazoles) towards poliovirus type 2 and have concluded that, although many compounds show purely inhibitory action, only 2-(α-hydroxybenzyl)-benzimidazole (II ; R_1 = Ph, R_2 = OH) and its 5-chloro derivative are potentially useful, in view of their selective action on the virus. As 2-hydroxymethylbenzimidazole (II ; R_1 = H, R_2 = OH) and the 2-(α-hydroxyethyl)-compound (II ; R_1 = Me, R_2 = OH) are both inactive, and as the 2-benzyl derivative shows slight activity, the presense of both the benzyl group and a suitably sited hydroxyl group are probably necessary for useful activity in this particular series.

As 1-methyl-4′:4′-dibutylisatin-β-thiosemicarbazone (III) at 30 μM concentration and 2-(α-hydroxybenzyl)-benzimidazole at 100 μM concentration have similar effects on poliovirus type 2 in tissue culture (Fig. 1), the structures were examined for comparable features. The infra-red spectrum[8] shows the presence of intramolecular hydrogen bonding in the isatin derivative (III), and intramolecular hydrogen bonds are also possible in the benzimidazole (IV).

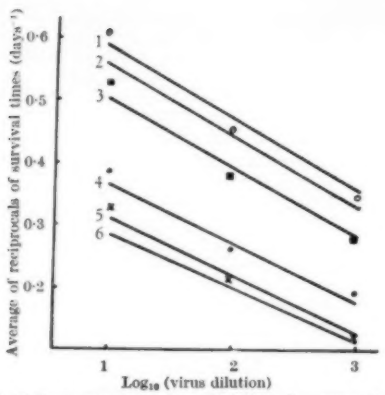


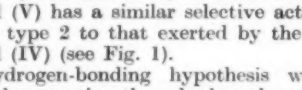
Fig. 1. Poliovirus type 2 assays. Variation of average reciprocals of survival times (in days) with logarithms of virus dilutions (in presence of 50 per cent of maximum tolerated dose of different compounds). (1) Control line without antiviral compound. (2) 2-(*o*-Hydroxyphenyl)-benzimidazole (10 μM). (3) 2-(*p*-Hydroxybenzyl)-benzimidazole (100 μM). (4) 1-Methyl-4′ : 4′-dibutylisatin-β-thiosemicarbazone (30 μM). (5) 2-(*o*-Hydroxybenzyl)-benzimidazole (50 μM). (6) 2-(α-Hydroxybenzyl)-benzimidazole (100 μM). For clarity, experimental points have been included only for line 3 (squares), line 4 (dots), line 5 (crosses), and for the control line (circles)

In the latter case, however, this bonding will be weak in view of the strain present in the small ring and in view of the strong tendency of benzimidazole derivatives to form intermolecular hydrogen bonds[9].

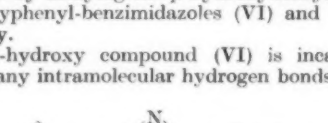
To shed further light on this, some related benzimidazoles were synthesized and tested. 2-(*o*-Hydroxybenzyl)-benzimidazole (V), which possesses the benzyl substituent and a hydroxyl group in the neighbourhood of the nitrogen atom, should form a similar, but slightly stronger, intramolecular hydrogen bond to that in compound (IV). Tissue culture experiments, described later, show that the *o*-hydroxy compound (V) has a similar selective action against poliovirus type 2 to that exerted by the α-hydroxy compound (IV) (see Fig. 1).

The hydrogen-bonding hypothesis was further examined by assaying the *p*-hydroxybenzyl- and the *o*-hydroxyphenyl-benzimidazoles (VI) and (VII) respectively.

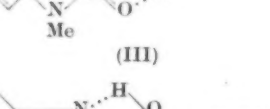
The *p*-hydroxy compound (VI) is incapable of forming any intramolecular hydrogen bonds, but can



(III)



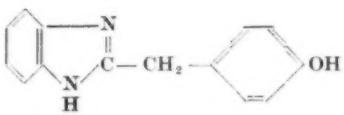
(IV)



(V)

associate by intermolecular hydrogen bonds. Bearing in mind the low activity of 2-benzylbenzimidazole itself, some activity might be expected in the *p*-hydroxy compound; but, if the existence of an intramolecular hydrogen bond is of importance, then the activity should be low. Fig. 1 shows that the activity of this compound is considerably less than that of the α-hydroxy compound (IV).

If the benzyl substituent were important for high activity in this series, then its absence from the *o*-hydroxyphenyl compound (VII) would affect the potential activity. This compound can, of course, form intramolecular hydrogen bonds of reasonable strength. Fig. 1 shows that the activity is less than that of the *p*-hydroxybenzyl compound (VI). This result indicates that replacement of the benzyl group by a phenyl group reduces activity, and, consequently, conjugation between the benzimidazole and benzene rings may be detrimental to the antiviral action.



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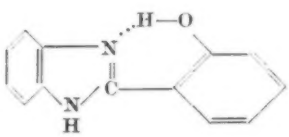

(VII)

Eggers and Tamm[10] have shown that the α-hydroxy compound (IV) acts by inhibiting virus multiplication. Now the presence of intramolecular hydrogen bonding is a feature common to all antiviral agents of high activity in both the isatin-β-thiosemicarbazone and the benzimidazole series. Thus it is probable that an active compound either chelates with a trace metal or forms intermolecular hydrogen bonds with some key molecule involved in the inclusion of nuclear DNA or perinuclear RNA into incomplete virus particles. Many simple benzimidazoles[9] and benzotriazoles[11] of lower activity[6] cannot form intramolecular hydrogen bonds, but have been shown by their infra-red spectra to form intermolecular N—H . . . N links. Strong, but non-specific, bonding to protein is possible with these compounds.

2-(*o*-Hydroxybenzyl)-benzimidazole was prepared by heating under reflux *o*-phenylenediamine (1 mole) and *o*-hydroxyphenylacetic acid (1 mole) with 2 *N* hydrochloric acid (2 moles) for 20 hr. The hydrochloride, melting point 130°, crystallized on cooling. After dissolving in water and making alkaline, 2-(*o*-hydroxybenzyl)-benzimidazole was obtained which, on crystallizing from aqueous ethanol, had a melting point of 229° (found: C, 74·7; H, 5·2; N, 12·1; $C_{14}H_{12}N_2O$ requires C, 75·0; H, 5·4; N, 12·5 per cent).

2-(*p*-Hydroxybenzyl)-benzimidazole was prepared in a similar way using *p*-hydroxyphenylacetic acid. After crystallizing from aqueous ethanol, it had a melting point of 270° (found: C, 74·8; H, 5·2; N, 12·1. $C_{14}H_{12}N_2O$ requires C, 75·0; H, 5·4; N, 12·5 per cent).

2-(*o*-Hydroxyphenyl)-benzimidazole, melting point 231°, was prepared by the method of Hübner[12], and 2-(*x*-hydroxybenzyl)-benzimidazole, melting point 201°, as described by Bistzycki and Przeworski[13].

Antiviral assays were determined in tissue culture experiments. Monolayers of *ERK* 1 cells[3] were grown in a number of tubes using *ELP* medium[3]. After three days, the media were replaced by otherwise identical media, but containing half the maximum tolerated dose of the test compound. Test compounds were absent from control tubes. Different dilutions (ten-fold) of the virus were added to the tubes, which were then rolled continuously at 37° and examined twice daily for signs of cytopathic effects. The end-point was taken when half the cells were affected. Average of reciprocals of survival time was plotted against the logarithm of the virus dilution (Fig. 1). Relative activities of compounds are given by displacement (to the left) of the line from that for the standard[14].

The results obtained indicate that both 1-methyl-4′:4′-dibutylisatin-β-thiosemicarbazone (III) and 2-(*o*-hydroxybenzyl)-benzimidazole (V) have activities against poliovirus type 2 which compare favourably with those observed with other compounds and 2-(*o*-hydroxybenzyl)-benzimidazole shows considerable promise as an agent against poliovirus type 2.

We thank Dr. A. P. Goffe, who kindly supplied the viruses (type 1 strain *Sc*, 2 *ab*; type 2 strain *P* 712, *Ch*, 2 *ab*) used in the assays, and Dr. F. W. Sheffield for his generous advice.

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RESPONSE OF CATFISH BARBELS TO TASTE STIMULI

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TASTE buds were first discovered and their function correctly interpreted by Weber[1] on carp, and it was not until 1867 that Schwalbe[2] and Loven[3] discovered independently similar taste organs on the tongues of mammals. Such taste-buds are found over certain areas of the skin of some fish, and are innervated by branches of the facial, glossopharyngeal and vagal nerves (Herrick[4]). In the catfish, these buds are abundant on the barbel, though they are also scattered over the whole body surface. Using behavioural techniques, Herrick[4] and Parker[5] studied the effect of applying chemical stimuli to the barbel, and confirmed that these buds are chemosensory organs. Later Hoagland[6] attempted to study chemoreception in the catfish electrophysiologically, but although he recorded activity from tactile fibres of the facial nerve innervating the barbel, he could record no spikes from the chemosensory fibres. However, he did detect an increase of neural activity through the audio-system. This suggests that, with the more refined techniques now available, responses from these fibres might be recorded, and in the present work this was attempted.

The freshwater catfish (*Ameiurus melas*) used weighed 200–300 gm. The fish was first anæsthetized by dipping into 0·03 per cent tricaine methanesulphonate solution for a few minutes, then transferred into 0·015 per cent tricaine solution and its head stabilized by a holder. In order to stimulate the nasal barblet and record electrophysiologically, the dorsal region of the head was exposed to air (Fig. 1). Only nasal barblets were used, since no response to chemical stimuli could be elicited from the maxillary barblet. After removal of the skin near the basal region of the barblet, the branch of the facial nerve to the barblet was isolated from the surrounding tissue and severed centrally. The nerve bundle was usually divided into small bundles, and one of them was placed on platinum electrodes to record the neural response. Both nerve and electrodes were covered with mineral oil, and the electrodes connected with an a.c. pre-amplifier, followed by a cathode-ray oscilloscope and electronic summator to quantify the neural response (Beidler[7]). The stimulating solution was allowed to flow slowly into a special chamber which surrounded the barblet. The barblet was washed with water after

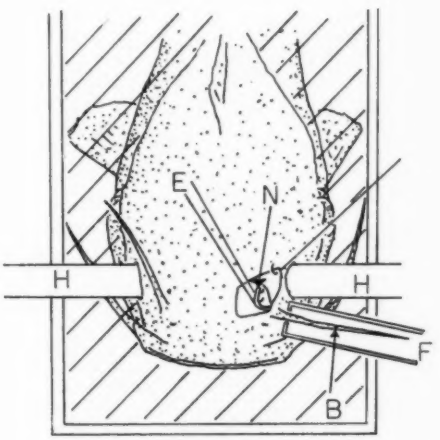

Fig. 1. Preparation for recording neural activity from the facial nerve in response to chemical stimulation; *B*, a nasal barblet; *F*, a flow chamber through which stimulating solution flows; *N*, a branch of the facial nerve; *E*, a pair of platinum recording electrodes; *H*, animal holder

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each stimulation. The responses to adequate concentrations of chemicals were reproducible for several hours, if the blood supply were intact.

Hoagland[6] assumed that spikes from the tactile fibres were larger than those from the chemosensory fibres. However, the present results show that many spikes caused by rapid flow of solution are of the same order of magnitude as those recorded from chemosensory fibres (Fig. 2), although some of them are larger. Since the rapid flow of solution causes a short burst of activity of such tactile fibres, only a gentle flow of solution was used.

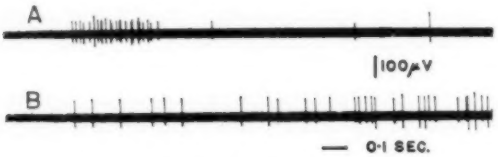

Fig. 2. Spikes from the facial nerve, which includes a few intact nerve fibres. *A*, responses of tactile fibres to rapid flow of tap water; *B*, responses of chemosensory fibres to 1 *M* sodium chloride. The record shown in *B* was obtained after cutting the tactile fibres in the nerve used for record *A*

Application of sodium chloride resulted in a train of spikes which continued throughout stimulation. The summated responses to various concentrations of sodium chloride are shown in Fig. 3, while in

Fig. 3. Summated response to tap water (*A*) and to sodium chloride of 0·1 *M* (*B*), 0·2 *M* (*C*), 0·25 *M* (*D*), 0·4 *M* (*E*), 0·5 *M* (*F*) and 1·0 *M* (*G*)

Fig. 4*a*, the responses are plotted against concentrations of various salts: sodium chloride, potassium chloride, lithium chloride and ammonium chloride. The maximum height of the integrated response to each stimulus was used as a measure of the magnitude of response. It is interesting to note that potassium chloride is more effective for catfish than is sodium chloride, which agrees with results obtained from carnivores, but is in contrast to those obtained from rodents (Beidler *et al.*[8]). The mean value of the ratio: $\frac{\text{response to 0·25 } M \text{ sodium chloride}}{\text{response to 0·25 } M \text{ potassium chloride}}$ is 0·36. Thresholds for these salts are about 0·1 *M* (Fig. 4*a*). This value is comparable with that of 0·1 *M* obtained behaviourally by Stieck[9] for the minnow and that of about 0·1 *M* obtained by Koketsu and Kimura[10] for the frog, but is higher than that of 0·002 *M* for the rat obtained by Beidler[7]. Divalent alkali salts of greater concentration than 0·5 *M* produce an irreversible reduction of activity, but the maximum magnitude of their response is not as large as that to potassium chloride. The effect of different anions was tested by using the sodium salts, sodium chloride, sodium bromide, sodium nitrate, sodium sulphate and sodium citrate. The magnitude of response to equi-normal concentrations of these salts was almost identical except the response to sodium sulphate, which was smaller than that to the other salts (Fig. 4*b*). Application of hydrochloric acid

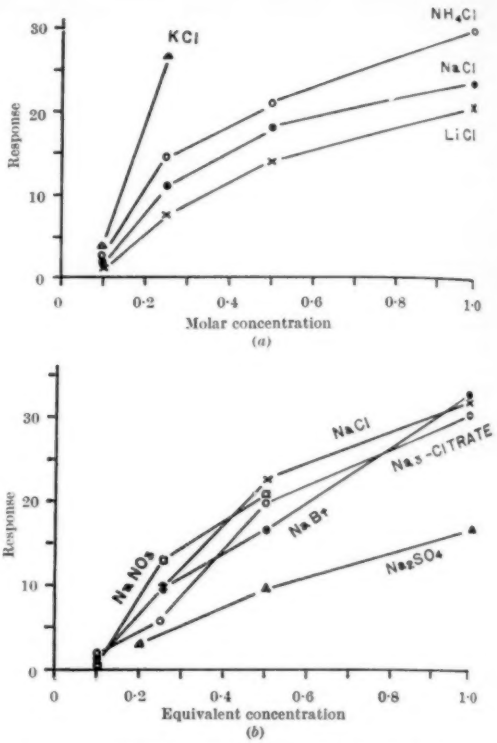

Fig. 4. *a*, relationship between summated response and concentration of chloride salts; *b*, relationship between summated response and concentration of sodium salts

elicited a large initial burst of impulses which declined rapidly until, within 10–20 sec. after application, almost no spike activity remained. The threshold for hydrochloric acid was about 0·0005–0·001 *M*, which is much higher than that of 0·00901 *M* obtained behaviourally by Allison and Cole[11] for the killifish, but is comparable with that of 0·001 *M* or less for the rat's tongue obtained electrophysiologically by me. Hydrochloric acid, 0·01 *M* or higher in concentration, caused an irreversible reduction of response. Maximum response to 20m*M* quinine hydrochloride was reached in ten or more seconds after application of the stimulus, and spike activity continued for a short time after washing the barblet with water, although no irreversible change in receptor activity was observed.

I am grateful to Dr. Lloyd M. Beidler for his kind suggestions and criticisms.

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LETTERS TO THE EDITORS

GEOPHYSICS

Diurnal Variation of High-latitude Auroral Frequency on Magnetically Quiet and Disturbed Days

DURING the winters of 1957–58 and 1958–59 an all-sky camera was operated by the Danish Meteorological Institute at Nord in Greenland (geographical co-ordinates 81·6° N., 343·4° E.; geomagnetic co-ordinates 80·9° N., 134·5° E.).

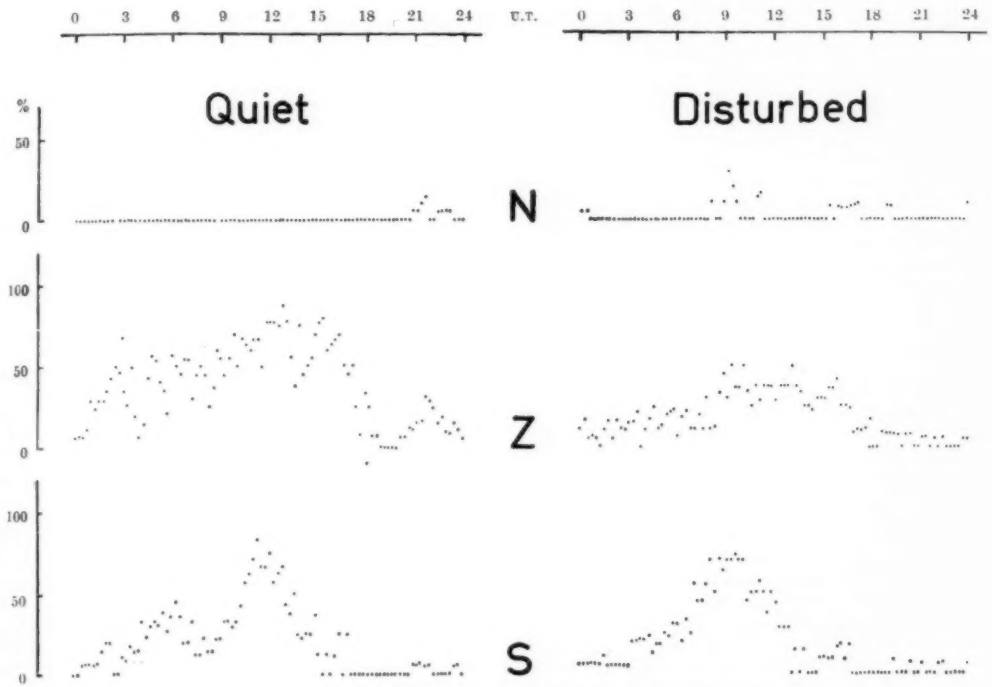

Fig. 1. Diurnal variation auroral frequency, Nord, 1957–59

Following a proposal by Stoffregen[1] the sky was divided into sectors north (N), between 80° and 60° northern zenith distance, zenith (Z), between 60° northern and 60° southern zenith distance, and south (S), between 60° and 80° southern zenith distance, north being defined here as the direction perpendicular to the auroral isochasm[2] through the station; geographically this direction is approximately west-north-west.

Fig. 1 shows the diurnal variation of auroral frequency in the three sectors on international quiet and disturbed days, respectively. It is seen that zenithal auroræ are most frequent in the day hours of international quiet days, with two principal maxima at about 5 and 13–14 hr. U.T. On international disturbed days these auroræ are less frequent. In S the quiet day variation similarly shows two distinct maxima, at about 6 and 11–12 hr. U.T., whereas the variation on disturbed days is of a different shape, with a single maximum at about 9 hr. U.T., that is, near magnetic noon in the Mayaud system[3]. Smaller peaks at 9 hr. may be seen in the diurnal variation on disturbed days at Z as well as at N.

It is proposed that the observation should be interpreted as follows:

At magnetic noon the auroral zone is situated not far to the south of the station. When on magnetically disturbed days the auroral incidence near the zone increases, the zone broadens and zonal auroræ are observed from the station near magnetic noon.

The auroræ which are observed above the station on quiet days become less frequent on disturbed days, possibly due to some sort of modulation effect. They may be said to belong to a special polar population (inner auroral zone), or, what may be the same in other words, to form a quiet-day appendix to the day-time part of the main auroral zone.

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ASTROPHYSICS

Megashatter Cone Hypothesis of the Origin of Lunar Volcanoes

I BELONG to the school that interprets the main observable lunar topographical features as the result of impacts with asteroids and comets. To proponents of this belief, a stunning blow was delivered in 1959 when Kuiper[1] exhibited, during a space symposium in Washington, his beautiful pictures of small features that just could not be impact explosion craters. The discovery of release of carbon dioxide gas also strengthened the picture of some internal lunar feature through which gas could be released.

Now another possibility occurs that the lunar volcanoes are not volcanoes at all, but extremely large shatter cones (which will be referred to as megacones or megamegacones) produced by the shock-waves resulting from impact of an asteroid. These would appear as stumps in the central uplifts of very ancient eroded lunar craters, namely, lunar astroblemes. Dietz[2] has found terrestrial shatter cones as large as 6 ft. long in the Kentland, Indiana, 'cryptovolcanic' structure. He proposed the term 'astrobleme' for features of the Kentland type.

It is proposed that megacones of extremely large size exist and that these are composed of the smaller groups of shatter cones discussed in refs. 3 and 4. It is proposed that the roots of a central uplift in a crater formed by a large impact explosion produced by a nickel–iron asteroid consist of a megamegacone in turn composed of many concentrically arranged megacones all originally pointing upwards and inward toward the centre of impact. Most of these will have been almost instantly blown outward leaving a centre region filled with explosion breccia and/or devitrified impact glass. If glass is present, it will undoubtedly contain small spherulites of nickel–iron vapour.

The rebound after impact will lift up this root structure a considerable distance; however, it would always remain below the original lunar surface. Hydrodynamic forces resulting from the melting of the original ground-mass under extreme pressure and plastic flow of breccia dust produced would also tend to force the cone structure upward and outward. This megamegacone structure would then consist of somewhat tilted megacones with vertically up-ended ground-mass and breccia filling the voids between megacones during the original uplift.

After a couple of billion years one would expect that erosion of the uncompressed shattered matter around the stronger megacones would be removed as dust by methods as proposed by Gold[5] for a few craters at least. Shock of later impacts might also tend to unearth the buried compressed megacones by scattering the enclosing dust and exposing the volcano-shaped structure. A crater-like centre might be present in the original megamegacone because of the upward and outward thrust of its megacone components and the shape charge type of ejection of matter from the final rest point of the asteroid. If the volcano-like lunar structures are megacones, then their geometry may be a clue to their mineralogical composition and to the general selenology of the Moon. If the subsurface features of the Moon are largely strained, compressed, cone-like buried structures, then the shallow seismic effects of these regions may be predicted by a study of similar terrestrial features. Sharp, shattered rocks would be expected to be present near megacones because of gradual flaking from the compressed material resulting in the well-known small shatter-cone shapes and dust. The shattered nature of lunar astroblemes would probably promote faster dust formation by action of the extreme variations of temperature during lunar day and night than by the radiation damage mechanism proposed in ref. 5.

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Sun–Earth Relations and Associated Periodicities

PERIODICITIES of less than 27 days in geophysical disturbance data have been investigated for many years, with inconclusive results, due to inherent difficulties in determining the statistical significance of the computations. However, there is now an increasing amount of evidence, arising from the examination of independent data samples, to support the existence of such periodicities. Some of the recent evidence is summarized here.

Auto-correlation of the 3-hourly magnetic planetary index a_p, during short moderately disturbed intervals in 1952, disclosed periods of from one to four days[1]. Moreover, the times of occurrence of magnetic impulses seemed to be closely connected with the amplitude of these periodicities, and with changes from one periodicity to another.

Ward[2] computed the variance spectrum of the daily C_t, K_p, and A_p indices for some portions of 1941–56, and found periods of $27/n$ days, with $n = 1, 2, 3, 4, 5$ and 6. No interpretation of the overtones of the 27-day period was given.

An examination has been made of the time separation, ΔT, between 817 magnetic impulses occurring during 1949–59. Intervals of up to 70 days were tabulated. The declining portion of the sunspot cycle, 1949–54, shows maxima in the number of occurrences $N(\Delta T)$ at about the period of solar rotation. Similar maxima do not occur in $N(\Delta T)$ during 1955–59. The same data were examined for higher frequencies, using a one-day running average. During 1949–54, in addition to the solar rotation maxima, other maxima in $N(\Delta T)$ occur at multiples of about 3½ days. Similar maxima during 1955–59 occur at multiples of about 3 days (Fig. 1). This periodicity in $N(\Delta T)$ was

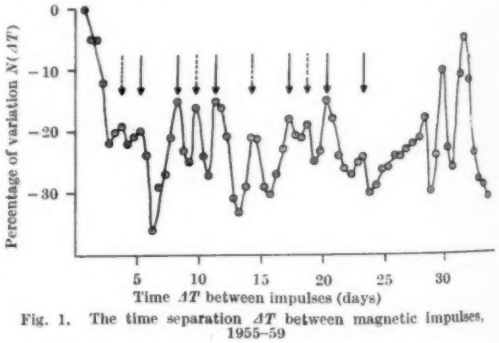

Fig. 1. The time separation ΔT between magnetic impulses, 1955–59

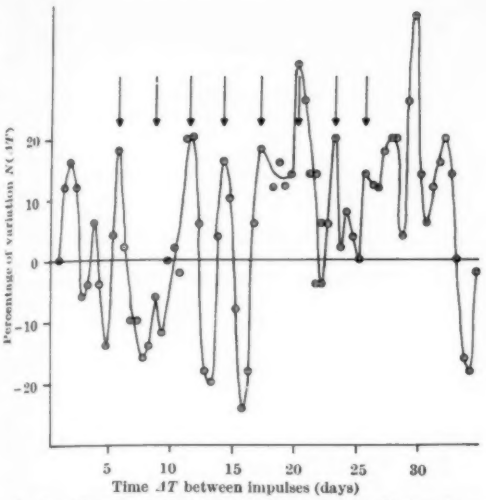

Fig. 2. The time separation ΔT between magnetic impulses during the International Geophysical Year

particularly strong during the International Geophysical Year, and the mean period was $2\frac{7}{8}$ days (Fig. 2). The data were insufficient to investigate periods of less than one day.

A separate investigation[3] was made of the time separation, Δt, between the occurrence of solar flares and magnetic *SSC* impulses during the International Geophysical Year. All flares listed, from 1⁻ to 3⁺, were given equal weight in the analysis. Intervals of up to 14 days were tabulated to the nearest $\frac{1}{4}$ day, from *SSC* to flare $(-\Delta t)$, as well as from flare to *SSC* $(+\Delta t)$. The $N(+\Delta t)$ curves show a $1\frac{3}{4}$-day periodicity which is not present in $N(-\Delta t)$, and which could conceivably arise from the transit time of a disturbance from Sun to Earth. Removal of the $1\frac{3}{4}$-day component leaves a 3-day periodicity in $N(+\Delta t)$ of about the same amplitude as a $2\frac{1}{2}$-day periodicity in $N(-\Delta t)$. It could not be determined from this analysis whether the above difference between $2\frac{1}{2}$ and 3 days is significant. A one-day periodicity in the International Geophysical Year flare listings[4] made it impossible to decide whether the $N(\Delta t)$ contain a non-spurious one-day component.

The above periodicities in the time separation between geophysical disturbance data may be significant in the interpretation of statistical examination of upper atmospheric disturbances. It was previously suggested[1] that the 1–4-day periodicities in a_p were consistent with repeated Sun–Earth transits of a hydromagnetic wave. Although this is still a possibility, it now seems probable that the observed periodicities are more directly related to the timing of solar disturbance events. The solar flare to *SSC* time analysis indicates that such solar disturbance events may occur within $\frac{1}{4}$ day of flare times, and this investigation is continuing.

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Possible Solar Flare Effects in the *F* Region of the Ionosphere

I HAVE read with interest the recent communication by Knecht and Davies on this subject[1] which reports a series of large solar flares accompanied by marked effects in the *F* region of the ionosphere. A particular feature is that the ionospheric effects and the flares are simultaneous only at isolated locations.

In this connexion, I should like to direct attention to a communication by Martyn, Munro, Higgs and Williams[2]. In that communication a correlation appeared to be established perhaps even more clearly than in the communication by Knecht and Davies. I write, however, not to claim priority of discovery but to report subsequent developments which considerably modified the conclusions in that communication. Continuation of that work brought to light two very significant new facts. It was found that the ionospheric disturbances occurred much more frequently than solar flares, and, even more important, that they almost always appeared at Canberra and Sydney with an appreciable time difference. They were, in fact, travelling disturbances. It becomes obvious, therefore, that there is a high probability, at any single point, of a disturbance occurring close to the time of a flare and an even higher one of a coincidence somewhere.

When the War began in 1939, much research work, including the above, ceased. Moreover, publication of scientific results was also largely inhibited until after the War, and ionospheric literature and history tend to start from that epoch, after a gap of some seven years.

These results, therefore, though reported locally to the Radio Research Board, were never published. When ionospheric work was resumed at this Laboratory in 1947 attention soon became directed to ionospheric travelling disturbances[3], and it was realized they were the same phenomena observed earlier.

The characteristics of such disturbances have now been very fully investigated here[4], and their manifestations are becoming more familiar in a variety of ionospheric investigations throughout the world, particularly as a result of work done during the International Geophysical Year. Their effects on ionosonde records are similar to those shown in Fig. 1 of Knecht and Davies's communication and they can also cause changes in frequency of the signals received by an ionospheric path as in their Fig. 2.

It would seem that since the ionospheric effects of a solar flare are due to ultra-violet radiation they should all be observed simultaneously over the sunlit surface of the Earth.

I would urge the authors, in their further work, to give very careful consideration to these aspects. For a correlation to have scientific value it must be significant both statistically and physically.

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Dr. Munro's scepticism regarding our recent suggestion[1] that certain large flares are associated with effects in the F region of the ionosphere is understandable. As he points out, a solar photon phenomenon of this type would be expected to be evident over the entire sunlit hemisphere and not only at one or two isolated stations. However, since the submission of our original communication, our work has revealed several new facts which may help to account for this apparent non-uniformity.

First, from further examination of vertical soundings data, it appears that F region flare effects sometimes do occur simultaneously at a number of sunlit hemisphere stations. The difficulty, of course, is to be able to determine with any degree of certainty whether or not an effect actually occurred in the presence of the normal variability of the F region and the flare-associated increase in D region absorption which usually is responsible for the loss of several crucial F region observations. These factors, coupled with the relative infrequency of large flares, are undoubtedly responsible for the fact that the F region flare effect phenomenon has been so infrequently recognized in the past.

The second new point concerns the height of the $F2$ layer just prior to the beginning of the flare. We have found a strong tendency for the F region solar flare effect to occur only at those locations where the height of the $F2$ maximum electron density level ($h_{max.}$) at the time of the flare is relatively low (less than about 315 km.).

The flare of November 12, 1960, has been chosen to illustrate these points. Fifteen-minute values of $F2$ layer penetration frequency ($foF2$) are shown for six sunlit sounding stations in Fig. 1. The stations are arranged in order of increasing h_{max} as determined by the manual 10-point method. The time at which the flare begins (1323 U.T.) is shown by an arrow on the time-scale. Considering pre-flare and post-flare trends, it seems clear that a flare-associated increase in penetration frequency, which is related to maximum electron density, occurred at at least four of the stations (San Salvador, Reykjavik, Belvoir and Talara). Flare-associated increases probably did not occur at Huancayo and Concepcion. Note that the magnitude of the flare effect discontinuity seems to vary inversely with h_{max}. This is what would be expected on the simple picture where the flare radiations have peak ionization-rates at a level significantly below the $F2$ layer, the rates falling off exponentially with increasing height above this level.

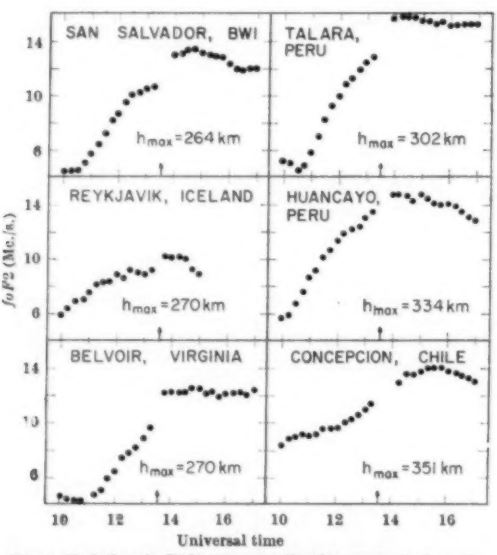

Fig. 1. Variations in $F2$ layer penetration frequency at six sunlit sounding stations during the large solar flare of November 12, 1960. Arrow marks flare beginning

Because of the simultaneity of the increases over distances of 10,000 km. or more, the possibility that the effects shown in Fig. 1 are due to travelling ionosphere disturbances is, it seems to us, extremely remote. Moreover, travelling disturbances can be rather conclusively eliminated as the cause of even some single station occurrences, like that shown in our earlier communication[1] for Adak, Alaska, during the flare of November 15, 1960, since, during the 2½-year period from July 1957 until December 1959, on only one day were occurrences of discontinuities in $foF2$ of this magnitude (1·5 Mc./s. increase in 15 min.) observed during the corresponding period (1200–1800 L.S.T.). To have one of the largest $F2$ layer travelling disturbances that occur at Adak pass over the station at almost exactly the same time as the occurrence of a large and relatively rare type of solar flare would seem fortuitous indeed. Also, according to the earlier work by Munro and Heisler[2], travelling disturbances always take the form of first a reduction in penetration frequency and then an increase whereas in the present phenomena the increase occurs first. Lastly, on the ionograms travelling disturbances are generally seen as additional cusps near the penetration frequency. Cusps of this type have not been observed on the ionograms following the solar flare events.

Miss R. E. McDuffie has contributed substantially to the work discussed in this communication.

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Interstellar and Interplanetary Communication by Optical Masers

In *Nature* of April 15, 1961, p. 205, reference is made to seven stars of nearly the same luminosity and spectral characteristic as our Sun within 10 light years. Apparently this erroneous statement is derived from an earlier general statement in *Nature*, September 19, 1959, p. 844, except that 15 light years was changed to 10 light years.

Even within 15 light years, there is only one star with "nearly the same luminosity and spectral characteristic as our Sun", namely, the brighter component of the Alpha Centauri system, while possibly one other may be considered to qualify, namely Tau Ceti, with a luminosity of 36 per cent of the Sun and spectral type $G4$ (instead of $G0$ for the Sun).

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Where the stars listed by Cocconi and Morrison are referred to in our article, 10 light years was erroneously written for 15 light years as noted by

Dr. van de Kamp. Even this is not strictly correct, since one of the distances is as large as 16·5 light years. But these differences are not crucial for our purposes.

The question whether certain stars are of "nearly the same luminosity and spectral characteristic as our Sun" is a more interesting one. A term such as 'nearly' can have no absolute meaning and must depend on a scale of measurement relevant to the discussion at hand. For example, it would not seem out of order for an astronomer to refer to stars within 15 light years distance as 'nearby'. But such description may be shocking to a pedestrian when he learns that their distance is many trillions of miles. In the case of our discussion, nearness or similarity should properly be measured in terms of what might be approximately equivalent for the possible development of life on planets somewhat similar to those we know. On such a scale, ranges of about two magnitudes in luminosity and with spectral types *G* and *K*, which cover the stars in question, seem fairly small. But it is understandable that Dr. van de Kamp, accustomed to contexts where a different scale of measurement may be appropriate, does not find our statement a natural one.

We hope that the actual list of stars, to which reference is given, leaves readers in no serious doubt about the facts.

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PHYSICS

Dependence of Thermo-stimulated Current Curves on Heating Rate

THE use of thermo-stimulated current and thermo-luminescent ('glow') curves, to determine the energy distribution and population of traps in insulators and semi-conductors, has recently been severely criticized[1] because of the interactions which are now known to occur between energy-levels in the forbidden gap. Nevertheless, it has so far been assumed that the shape of such curves is only slightly dependent on the heating-rate in the specimen.

During observations of thermo-stimulated currents in a number of cadmium sulphide single crystals following irradiation at 90° K. with cobalt-60 γ-rays, we have noted in one specimen a current peak, the shape of which is critically dependent on the rate of temperature increase. As shown in Fig. 1, the thermo-stimulated current curve generally exhibited two peaks corresponding to trapping-levels at 0·09 eV. and 0·13 eV. determined using the formula of Grossweiner[2]. For rates of rise in temperature between approximately 17° C. per min. and 19° C. per min. a very marked increase occurred in the amplitude of the peak corresponding to the deeper trapping-level, the increase appearing as a secondary peak of high resolution. No significant changes in the shapes of the two main peaks were observed when the secondary peak was present.

If Grossweiner's formula[2] is applied to the secondary peak, the derived energy-level is 1·0 eV., which is below the steady-state Fermi-level calculated from the dark current at room temperature.

It seems clear that monomolecular and simple bimolecular models on which theories of 'glow' curves have been based in the past[3,4] do not account for the present observation; but it is reasonable to assume that the high peak is associated with a particular energy-level of traps, which is initially filled and may empty either directly into the conduction band or indirectly through a second energy-level which is initially empty.

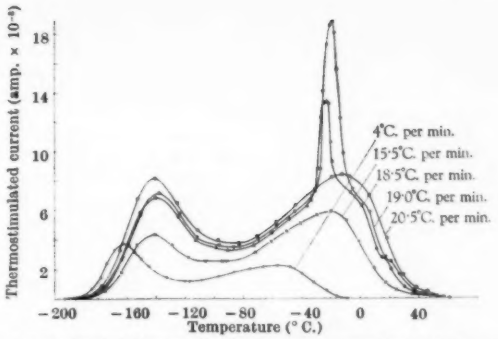

Fig. 1. Typical thermo-stimulated current curves for a cadmium sulphide single crystal exhibiting a secondary peak highly sensitive to heating-rate

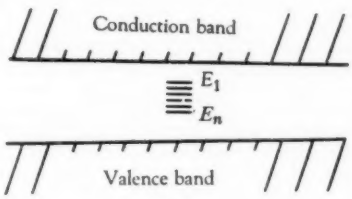

Fig. 2

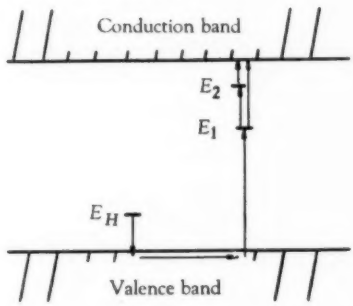

Fig. 3

To account for the observation two possible models, shown in Figs. 2 and 3, are suggested.

In the first model, Fig. 2, electrons are assumed to be held in traps of increasing depth E_1 to E_n. It is also assumed that the escape frequency factor increases with depth of trap. At low heating rates electrons are released directly into the conduction band from E_1 to E_n in turn, giving a broad thermo-stimulated current peak. At high heating rates electrons are released from E_n to E_1 in turn, again giving a broad peak. For particular values of trap depths and escape frequency factors, a high degree of simultaneous release may occur for a narrow range of intermediate heating rates resulting in a current peak of the type observed.

In the second model, Fig. 3, electrons associated with the appearance of the anomalous peak are assumed to be held in traps at the level E_1, and may be

released either directly into the conduction band or indirectly through the second level E_2 with which E_1 is spatially associated. The probabilities of direct or indirect release from E_1 to the conduction band depend on the heating rate and the escape frequency factors for the two levels.

If the temperature of the crystal is raised slowly, the peak corresponding to E_1 does not appear since the electrons are all released indirectly through E_2.

At some higher rate of rise of temperature the values of the escape frequency factors allow electrons from E_1 to be released directly into the conduction band giving a peak of the type observed.

To account for the disappearance of the peak at higher heating rates, a hole trap, E_H, is introduced and E_1 is also assumed to act as a recombination centre.

The escape frequency factor for holes in E_H is such that, for heating rates below that corresponding to the appearance of the anomalous peak, the holes are released after the release of the electrons from E_1. At higher heating rates, the holes, which are now released first, recombine with electrons at E_1 so that the anomalous peak no longer occurs.

The accurate evaluation of energy-levels from thermo-stimulated current and 'glow' curves depends on a satisfactory model being available. Observations such as the present, and those reported by Hoogenstraaten[1] and by Halperin and Braner[4], indicate the limitations of the method and the care which must be exercised in the interpretation of results.

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Electrical Conductivity of Calcium Oxide at High Electric Fields

Interest in the study of the electrical properties of the alkaline earth oxides as an aid to understanding the physics of the oxide-coated cathode has led to an extension of work previously carried out on the electrical conductivity of calcium oxide at low electric fields[1]. In order to obtain activation energies for the conduction processes[2], it is necessary to determine the conductance at zero applied field as a function of temperature; but information of some value can be obtained from a study of the conductance of the cathode material at higher electric fields than those commonly employed. Reports of investigations at high fields are much less numerous than those of conventional measurements, but the work of Danforth and Goldwater[3] and of Nakai, Inuishi and Tsung-Che[4] may be referred to. Of interest is the recent investigation by Adams and Jacob[5] on strontium oxide: the behaviour of its high-field conductance appears to be dissimilar to that of barium oxide, the form of the graph of conductance versus applied voltage depending strongly on the temperature of the oxide. The present work on calcium oxide may be of interest in a general comparison with results from the other alkaline earth oxides.

Two types of experimental tube have been used in the present work: the calcium oxide samples were contained between nickel or between platinum cathode bases. The construction and processing of the tubes have been described in a previous communication[1]. Conductivity currents were measured on a galvanometer for applied potentials of up to 100 V. d.c., and characteristics of conductivity current versus applied potential were traced at various values of the sample temperature. The conductance of the calcium oxide sample at an applied potential V was determined by taking the gradient of the characteristic at the relevant value of V.

Fig. 1 shows a typical series of current-voltage characteristics for a nickel-based sample of calcium oxide for the range of temperature T of 598–926° K., and Fig. 2 shows the variation of the conductivity with the applied voltage for these curves. Currents in the voltage- and temperature-ranges shown were stable, but at temperatures greater than about 1,000° K. for this sample the application of potentials greater than 10 V. was sufficient to cause gross instabilities due to breakdown. Curves of similar form were obtained with platinum-based samples, although conductivity currents were smaller as the activation process was less effective. For the sample giving the results in Figs. 1 and 2, the conductivity plot obtained by plotting $\log \sigma_0$ (where σ_0 is the zero-field conductivity) versus $1/T$ shows two approximately linear portions intersecting at about 700° K.; above this temperature the Loosjes–Vink hypothesis[2] predicts that the current-voltage characteristics should be curved towards the applied potential axis, and below it they should be linear. Such a difference in the form of the characteristics is, in fact, observed. The general form of the variation of conductivity with applied potential in the high-temperature region is broadly the same as that found by Danforth and Goldwater[3] in pulsed current measurements on barium oxide, strontium oxide and the mixed oxide: the

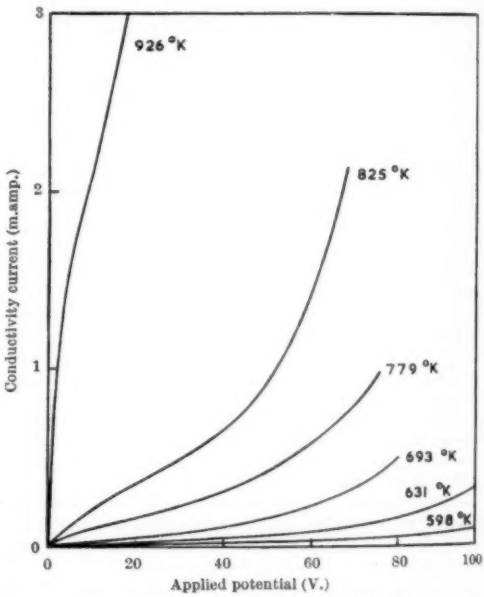

Fig. 1. Conductivity current–voltage characteristics for a sample of calcium oxide

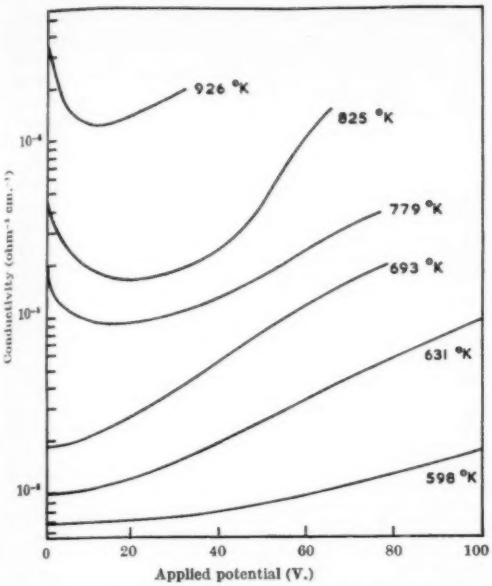

Fig. 2. Conductivity as a function of applied voltage for calcium oxide

conductivity decreases to a minimum and then increases with increasing field. An interesting feature of the characteristics is the increase in conductivity at high fields, which is observed throughout the temperature range. Curvature of the current-voltage characteristics in this sense might be expected from a consideration of the Joule heating effect of the conductivity current, but an estimate of the orders of magnitude of the quantities involved shows that the curvature cannot be attributed solely to a heating effect. In the range of temperatures above 700° K., where for this sample the conduction process is predominantly one of thermionic emission across the pores of the oxide, the increase of conductivity current with applied voltage is too rapid to be completely accounted for by the Schottky effect. The fact that similar curves are obtained for nickel- and platinum-based samples indicates that the form of the characteristics is a property of calcium oxide rather than of any interface compound which may be formed between calcium oxide and the nickel cathode base.

A detailed comparison between the results of different workers using the various oxides is not possible. Among the difficulties involved is a satisfactory definition of the field across the porous specimen[5], and the possibility also exists of differences in the porosities of samples used by different workers, which may cause considerable differences in the shape of the characteristics[3]. The general conclusions reached as a result of the present work are that at low electric fields the shape of conductivity current-voltage characteristics for calcium oxide agrees with the predictions of the Loosjes–Vink hypothesis; at high fields an increase in conductivity is observed, similar to that previously observed in the other alkaline earth oxides and in the mixed oxide[3]; and the field- and temperature-dependent effects obtained with strontium oxide[5] are not found with calcium oxide in the temperature-range investigated.

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A Difference Band of a Fundamental and a Lattice Mode in the Infra-red Spectra of Lead and Other Carbonates

Lead carbonate, $PbCO_3$, crystallizes in the potassium nitrate structure, space group D_{2h}^{16}, with four molecules per unit cell[1]. From crystallographic considerations, it follows that the carbonate ion occupies a site in the lattice of which the symmetry is C_s. The degeneracies of ν_3 and ν_4 are removed by the perturbing crystalline field, and all the vibrational modes are active in the infra-red.

'Vaseline' mulls of lead carbonate were prepared and the spectra were recorded with a Perkin–Elmer model 21 spectrometer fitted with sodium chloride optics. The frequencies and assignments are given in Table 1 and the infra-red spectrum is shown in Fig. 1.

Table 1. Frequencies and Assignments of the Infra-Red Bands of Lead Carbonate

Frequency (cm.$^{-1}$)	Relative intensity	Assignment
2,405	vw.	$\nu_1 + \nu_4$
1,731	w.	$\nu_1 + \nu_3$
1,437	vs.	ν_3
1,406	vs.	ν_3
1,053	m. vsp.	ν_1
849	s. sp.	ν_2
824	vw.	ν_2-lattice mode
690	sh.	ν_4
678	s.	ν_4

In agreement with the prediction based on the crystal structure, the degeneracies of ν_3 and ν_4 have been removed and the infra-red inactive ν_1 has become infra-red active.

A small band occurs on the low-frequency wing of ν_2 of the spectra of the following salts:

Lithium carbonate (work in this Laboratory)			Sodium nitrate (ref. 2)		
Sodium	,,	,, ,,	Lithium	,,	(work in this Laboratory)
Potassium	,,	(ref. 2)	Potassium	,,	,, ,,
Lead	,,		Calcium	,,	,, ,,
Calcium	,,	(ref. 2)	Barium	,,	,, ,,
			Strontium	,,	,, ,,

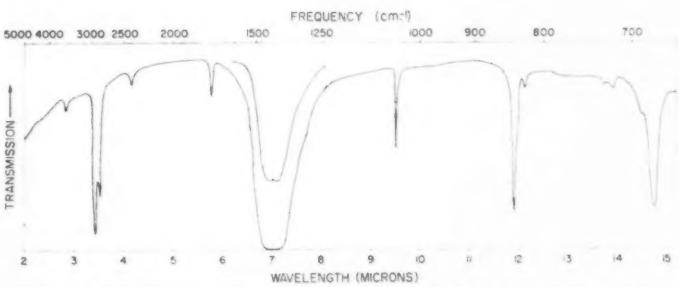

Fig. 1. Infra-red spectrum of lead carbonate ('Vaseline' mulls). ν_4 is shown in detail with the 'Vaseline' bands compensated for by a 'Vaseline' sample in the reference beam of the spectrometer

The frequency difference between ν_2 and the small band is always of the order 10–30 cm.$^{-1}$. An explanation of this phenomenon can be given on the following lines. The ν_2 mode of planar XO_3^- ions can be described by a motion in which the central X atom moves perpendicular to the plane of the oxygen atoms. This corresponds to movements in two directions in the crystal if the crystal contains a two-fold axis, and in one direction if a three-fold axis is present. This directional movement is only present in the case of ν_2. It is evident that this situation greatly facilitates the transfer of energy between the ν_2 vibration and a translational lattice mode. The result of this coupling is that a difference band appears of ν_2 and a translational lattice mode. From these considerations it can be predicted that this difference band occurs in the infra-red spectra of all crystals containing flat XO_3 ions.

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CHEMISTRY

Products of the γ-Radiolysis of Aqueous Cystine Solutions

Recently, Markakis and Tappel[1] determined the yields of cysteine, alanine, ammonia, hydrogen sulphide, sulphur and sulphate in hydrochloric acid solutions of cystine irradiated with γ-radiation from spent fuel elements. However, their results indicate that especially at low doses ($< 10^6$ rads) not all the radiolytic products were identified. The present communication describes the identification of products in cobalt-60 γ-irradiated unbuffered and acidified cystine solutions at relatively low doses.

All solutions were made using doubly-distilled water and L-cystine (Mann-assayed grade from Mann Research Laboratories, Inc. $[\alpha] = -216°$ in M hydrochloric acid). Oxygenation and deoxygenation of solutions were effected by bubbling oxygen and nitrogen respectively through the solutions during irradiation. All irradiations were carried out at ambient temperature with a dose-rate of about 670 rads/min. In order to inhibit cysteine oxidation 1 ml. of 0·1 M N-ethyl-maleimide (NEM) was added to each solution (50 ml.) after irradiation. Cystine solutions (3×10^{-4} M) were concentrated (25 fold) at 30–32° C. prior to spotting on paper for descending chromatography or electrophoresis. Radiolytic products were detected on developed chromatograms and electrophoretograms using the polychromatic copper nitrate–ninhydrin reagent[2].

In most cases excellent separation of ninhydrin-positive products was obtained using ethanol/water/sulphuric acid (80 : 20 : 0·5) for chromatography. Although development with ethanol/water (80 : 20) and butanol/acetic acid/water (4 : 1 : 5) also yielded fair separation of products, the use of a strongly acidic solvent seemed desirable in light of the ease of hydrolysis of cystine disulphoxide. The chromatographic data and spot colours (Table 1) suggest that cystine disulphoxide, cysteic acid, cysteine sulphinic acid, alanine and cysteine are radiolytic products.

Although the reaction between NEM and thiols is known[3] to proceed more slowly the lower the pH, the 'uncoupled cysteine' spot was not observed in the chromatograms of acidified cystine solutions provided they were allowed to react with NEM for at least one day prior to spotting. The presence of cysteic and cysteine sulphinic acids in unbuffered solutions was confirmed by paper electrophoresis at a number of pH values. Some confirmation of the identification of cystine disulphoxide in certain irradiated solutions follows from the observation that the 'disulphoxide spot' was absent in chromatograms of aliquots (containing no NEM) to which cysteine had been added prior to chromatography. This is to be expected since it has been shown[4] and confirmed in the present work that cysteine reacts with cystine disulphoxide to form cystine and cysteine sulphinic acid.

No precise quantitative analysis of the products was attempted and the very approximate G-yields listed (Table 2) were deduced from visual comparison of the spots from irradiated solutions with those from appropriate amino-acid solutions.

In hydrochloric acid solutions estimation of the amounts of cysteic acid on chromatograms developed with the ethanol/sulphuric acid solvent was not possible owing to interference from a grey-blue spot with R_A of 0·7. However, fair separation of the cysteic acid/cysteine sulphinic acid fraction from other species present was effected with ethanol/water (80 : 20). When this solvent was used, the irradiated solutions were first dried to remove hydrochloric acid and redissolved in 10^{-2} M acetic acid prior to spotting on paper.

Table 1. R_A (R_F rel. to Alanine) Values of Radiolytic Products from Cystine

Compound	R_A* and (colour)†	R_A* and (colour)† of spot from irradiated solution
Cystine disulphoxide	0·41 (g–b)	0·38 (g–b)
Cystine	0·54 (g)	0·54 (g)
Cysteic acid	0·65 (p)	0·65 (p)
Cysteine sulphinic acid	0·80 (p)	0·78 (p)
Alanine	1·00 (p)	1·00 (p)
Cysteine (uncoupled with NEM)	0·94 (g)	0·94 (g)
Cysteine (coupled with NEM)	1·05 (r)	1·04 (r)

*, in EtOH/H_2O/H_2SO_4 (80 : 20 : 0·5).
†, g = grey; b = brown; p = purple; r = rust.

Table 2. Products of Irradiated 3×10^{-4} M Cystine Solutions (Dose of 8×10^4 rads)

[Acid]	Initial degree of oxygenation*	Products† and (approx. G-yield)			
Unbuffered	O	RSO_2SR‡	RSO_2H (1·1),	RSO_3H‡	RSH‡	RH‡
Unbuffered	A		RSO_2H (1·2),	RSO_3H‡	RSH (0·9),	RH‡
Unbuffered	D		RSO_2H‡	RSO_3H (0·1),	RSH (0·9),	RH‡
0·04 M HCl	O	RSO_2SR‡	RSO_2H (0·3)	RSO_3H‡	RSH (0·5),	RH‡
0·04 M HCl	A		RSO_2H (0·3),	RSO_3H‡	RSH (0·6),	RH‡
0·04 M HCl	D			RSO_3H‡	RSH (0·7),	RH‡
0·02 M H_2SO_4	O	RSO_2SR (0·4),	RSO_2H (1·5),	RSO_3H‡		RH‡
0·02 M H_2SO_4	A	RSO_2SR‡	RSO_2H (1·5),	RSO_3H‡	RSH (0·4),	RH‡
0·02 M H_2SO_4	D		RSO_2H‡	RSO_3H‡	RSH (0·5),	RH‡

* O, Oxygenated; A, aerated; D, deoxygenated.
† RSO_2SR, Cystine disulphoxide; RSO_2H, cysteic acid; RSO_3H, cysteine sulphinic acid; RSH, cysteine; RH, alanine.
‡ G-yield < 0·1.

Discussion of the results is simplified by assuming that the following reactions occur:

$$RSSR + OH \rightarrow RSOH + RS\cdot \quad (1)$$
$$RSSR + H \rightarrow RSH + RS\cdot \quad (2)$$
$$RS\cdot + RS\cdot \rightarrow RSSR \quad (3)$$
$$RS\cdot + X \xrightarrow{O_2} RSO_2SR + Y \quad (4)$$
$$H + O_2 \rightarrow HO_2 \quad (5)$$
$$RSH + RSO_2SR \rightarrow RSO_2H + RSSR \quad (6)$$
$$RSH + RSOH \rightarrow RSSR + H_2O \quad (7)$$

The sulphenic acid formed in (1) is assumed to be oxidized by hydrogen peroxide (or HO_2) through the sulphinic acid to cysteic acid. Reactions (2) and (7) would explain the low yields of cysteic acid in deoxygenated solutions. In aerated solutions apparently oxygen is depleted to such an extent (preliminary experiments indicate that in unbuffered aerated solutions 18 per cent of the initial oxygen content remains after 80,000 rads) that some hydrogen atoms are available for reaction (2). Reaction (6) helps to maintain high yields of cysteic acid in aerated solutions and, of course, ensures that high yields of cysteine and cystine disulphoxide are never detected in the same irradiated solution. The low yields of cystine disulphoxide and cysteic acid in hydrochloric acid solutions is presumably in part due to chloride ion-inhibition of reaction (1). However, the mechanism of the inhibition must remain obscure until the grey-blue spot observed on chromatograms of irradiated hydrochloric acid solutions of cystine is identified. The low yield of cystine disulphoxide in unbuffered solutions is not unexpected owing to rapid hydrolysis of the disulphoxide in these solutions. Although the accuracy of the yield-estimates is poor, the results seem to indicate that yields may depend on the *p*H of the solutions. It is possible that the observed increase in yield of cysteine and the decrease in yields of cysteic acid in unbuffered solutions is due to participation of the O_2^- ion which could conceivably reduce cystine to cysteine.

Regarding the possible effect of dilute hydrogen peroxide on cystine, it has been found that chromatograms of cystine (3×10^{-4} *M*)–hydrogen peroxide (2×10^{-4} *M*), which had been concentrated in the normal manner, exhibited only one faint spot with R_A characteristic of the disulphoxide.

We are indebted to Mr. A. Casselman and Dr. R. Greenhalgh for advice on electrophoresis and chromatography.

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Inhibition of the Formation of 3,4-Benzpyrene during Hydrocarbon Combustion

It is well known that polycyclic hydrocarbons including the carcinogen 3,4-benzpyrene occur in association with the carbonaceous deposits or smokes resulting from the incomplete combustion of a wide variety of organic materials. Thus 3,4-benzpyrene has been identified in soots, carbon black, certain smoked foods, cigarette- and tobacco-smoke and in the exhaust smoke of Diesel engines. The mode of formation of 3,4-benzpyrene has been reviewed by Badger *et al.*[1], who have suggested possible intermediates during the pyrolysis of organic compounds, and have pointed out that the observed products can be accounted for by secondary reactions involving initial or primary free radicals. A recent examination of the products from the pyrolysis of petroleum hydrocarbons by electron spin resonance supports the view that free radicals participate; the phenalenyl radical[2] has been identified.

The subject of formation of carbon in flames has attracted attention in recent years; the mechanism is still obscure but polycyclic hydrocarbons are often associated with the carbonaceous material formed. The present work was undertaken to determine whether the formation of 3,4-benzpyrene can be inhibited during hydrocarbon combustion. It is well established that nitric oxide exerts a remarkable inhibiting effect on the chain-decomposition of some hydrocarbons[3]. A substance was sought which would give rise to nitric oxide as a product of pyrolysis. It is known[4] that the carbon zone in a diffusion flame is a region where the hydrocarbon is decomposed thermally in the absence of oxygen (although OH radicals do reach this zone) and it seemed plausible that the production of nitric oxide within this zone might lead to inhibition of free-radical reactions leading to polycyclic hydrocarbons. Grey *et al.*[5] have investigated the thermal decomposition of nitroalkanes and have found nitric oxide in the products (with formaldehyde and some nitrogen dioxide, etc.).

In the present work, known amounts of nitroethane were introduced into a stream of commercial propane ('Bottogas') which was burned as a laminar diffusion flame in air. The air supply was reduced to give heavy soot formation in the absence of additive and the soot was collected, mainly on a brass target placed just above the flame, but also from the walls of the surrounding 'Pyrex' chimney. A weighed amount of

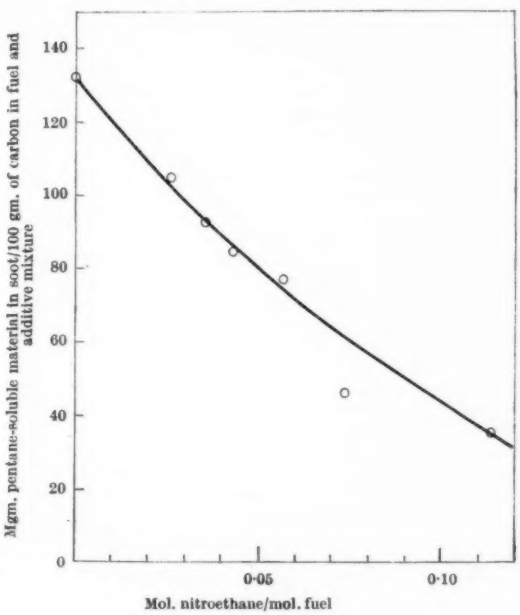

Fig. 1

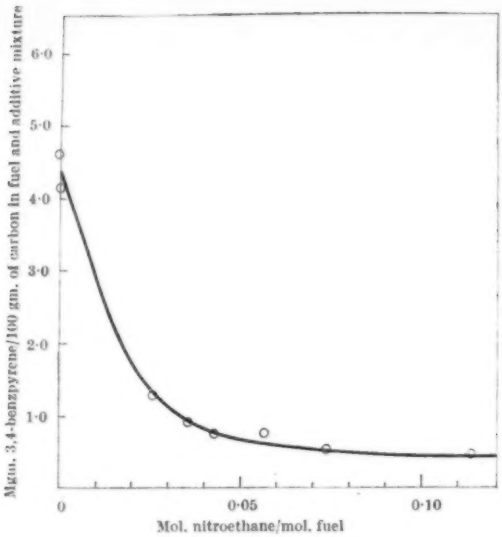

Fig. 2

soot was extracted with A.R. chloroform in a Soxhlet apparatus for about 3 hr. The solvent was removed by distillation in an atmosphere of nitrogen and the residue was extracted repeatedly with *n*-pentane. The extract was filtered and the *n*-pentane removed in an atmosphere of nitrogen; the residue was weighed and reported as mgm. *n*-pentane-soluble material per 100 gm. of carbon input in the fuel + additive mixture (Fig. 1). The 3,4-benzpyrene content of the *n*-pentane-soluble material was determined by the method described by Sawicki *et al.*[6]. This depends on the fact that 3,4-benzpyrene in a concentrated solution of sulphuric acid exhibits a characteristic peak in its fluorescence spectrum at 545 mμ when irradiated by ultra-violet light of activating wave-length 520 mμ. This peak is unaffected by the presence of other polycyclic hydrocarbons. The fluorescence spectrum was obtained by means of an Aminco-Bowman spectrophotofluorometer. In order to eliminate the effect of concentration quenching, the concentration of total polycyclic aromatic hydrocarbons was kept constant in each determination at 50 μgm./ml. Calibration was effected using a pure sample of 3,4-benzpyrene. It can be seen (Figs. 1 and 2) that small additions of nitroethane lead to a marked reduction in the formation of *n*-pentane-soluble material in the soot and in the amount of 3,4-benzpyrene.

Nitro-compounds (including nitroethane) are known to raise the cetane number when added in small amounts to Diesel fuels[7]. Although the present work has not been extended to engine studies we suggest that a reduction in emission of 3,4-benzpyrene from Diesel engines might be expected when nitroethane and similar additives are employed.

It has been found by a number of workers and recently confirmed by Pichler and Deschner[8] that no 3,4-benzpyrene is present in soot-free Diesel exhaust gases but under conditions of low revolutions and high load it does occur in the exhaust smoke. Although Diesel engines are probably relatively minor contributors to atmospheric pollution compared with industrial and domestic fuel-burning equipment[9], any attempt to reduce the emission of 3,4-benzpyrene in Diesel exhaust smoke, either by attention to engine design and maintenance or by chemical means, would be valuable.

We are indebted to the Coal Tar Research Association for the gift of a pure sample of 3,4-benzpyrene and to the Department of Surgery of the Medical School for permission to use the Aminco-Bowman spectrophotofluorometer.

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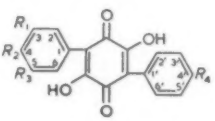
A Novel Type of Antioxidant related to Polyporic Acid

In the inhibition of the autoxidation of 1,4-dienes, for example, linoleic acid and its esters, it is generally assumed that polyphenolic compounds act as chain breakers in accordance with the mechanism $RO_2^{\cdot} + AOH \rightarrow ROOH + AO^{\cdot}$, where $RO_2^{\cdot}$ is the chain propagating peroxy radical and AOH is the antioxidant. The efficiency of the latter is determined not only by the rate of the inhibitory reaction but also by the stability of the intermediate $AO^{\cdot}$ radical[1]. Recently it was shown that certain polyhydroxy flavones[2] exhibit a remarkably high antioxidant efficiency. In this work, which was carried out with the view of finding better antioxidants for fat-containing foods, attention was concentrated on a group of compounds which were related to certain terphenyl quinones occurring in some edible fungi. They can be represented in terms of the following structure:



The compounds which were investigated include polyporic acid (R_1, R_2, R_3, R_4 = H), atromentin (R_1, R_3 = H; R_2, R_4 = OH) and leucomelone (R_1, R_2, R_4 = OH; R_3 = H), which occur in certain fungi and lichens. (A detailed discussion concerning the structure of leucomelone will be presented elsewhere.)

In addition, a number of related compounds which do not occur naturally were synthesized. They include trihydroxypolyporic acid (R_1, R_2, R_3 = OH; R_4 = H) and its *tert.*-butyl derivative (R_1, R_2, R_3 = OH; R_4 = *tert.*-butyl).

The synthesis of leucomelone has been described by Akagi[3], and in this investigation a similar method was used for the synthesis of the compounds mentioned above.

In the preparation of the new trihydroxypolyporic acids the reaction of 2,5-dichlorobenzoquinone with the appropriate *N*-nitrosoacetanilide gave 2,5-dichloro 3-phenyl 1,4-benzoquinone and 2,5-dichloro 3-(4-*t*-butylphenyl) 1,4-benzoquinone, which were then treated with diazotized 3,4,5-trimethoxyaniline. The dichloroterphenyl quinones thus prepared were hydrolysed to the corresponding dihydroxyquinones. Demethylation of the dihydroxy terphenyl quinones with a mixture of hydrobromic acid and acetic acid gave the pentahydroxyquinones directly; reductive demethylation followed by oxidation was not attempted.

The antioxidant activity of the compounds was tested in pure linoleic acid and in pure methyl linoleate at a temperature of 25° C. Uptake of oxygen was followed with conventional Warburg apparatus and this was correlated with peroxide formation, which was determined by means of an iodometric method[4]. A special test was used for the determination of antioxidant efficiency in the presence of metal catalysts, using ferrous phthalocyanine in ethyl benzoate solutions as standard. The results indicated that polyporic acid had no significant antioxidant activity, while atromentin and leucomelone were comparable with conventional antioxidants, for example, propyl gallate, for the inhibition of fat autoxidation. The new antioxidants 3,4,5-trihydroxypolyporic acid and 4′-*tert.*-butyl 3,4,5-trihydroxypolyporic acid showed outstanding activity. In linoleic acid these compounds gave protection factors which were, respectively, about ten and twenty-five times that obtained with propyl gallate. It should be noted that the *tert.*-butyl substituent substantially increases the solubility of the trihydroxypolyporic acid in higher fatty acids and their esters.

Anti-leukæmic activity of unsubstituted polyporic acid was reported recently[5]; it would be interesting to compare the polyhydroxypolyporic acids which show greater antioxidant activity.

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Ionic-Covalent Bonding in Crystals

An attempt[1] to resolve misunderstandings regarding ionic-covalent bonding in solids has been criticized in a recent article by Mooser and Pearson[2]. It should be pointed out, however, that their discussion of formal ionicity is limited to the simple and well-known case of atoms which each contribute one electron to a two-electron bond, and cannot be applied as it stands to 'dative bond' systems (such as are found in the zinc blende structure and in many other types of crystal, as well as in co-ordination complex molecules). In such systems formal covalency, defined as a state in which bonding electrons are equally shared between bonded atoms, can only be achieved by a donor–acceptor relationship with associated charge separation.

The main point in my communication[1] was the importance of the neutral-bond state (defined as that with zero charge separation) as the logical zero for the scale of electronegativity. (It should be noted that this is generally applicable; thus it holds for bonds in diatomic molecules, since for such molecules neutral-bonded and formally covalent states are identical.) This suggests that 'ideal covalency' (so termed for want of a better name) is associated with zero charge separation rather than with equal sharing of electrons. If this is so, the difficulty pointed out by Mooser and Pearson regarding the zero of the scale of bond ionicity used in my communication[1] can readily be resolved. It may well be that some revision of the accepted definition of the covalent bond on these lines is needed. However, in terms of the definitions used, the conclusions regarding charge separation presented in ref. 1 are still valid. In this connexion the concluding statement by Mooser and Pearson that "any attempts such as have been made to determine effective charges from formal bond ionicities are bound to fail" is certainly not a dogmatically required belief. It is my opinion, on the contrary, that useful qualitative information regarding charge separations in crystals can be obtained, although, for obvious reasons, some of which are referred to in my earlier communication, no quantitative estimates are as yet possible.

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Goodman's comments on our recent article[1] in no way affect our views on the subject of ionic-covalent bonding as outlined there. Since it is obvious, however, that Goodman is misinterpreting these views, we should like to stress once again the following points:

(1) The 'effective bond ionicity' is defined in terms of the actual charges associated with the constituents of a compound. It is, therefore, a physical quantity which can, at least in principle, be measured.

(2) The 'formal bond ionicity' is a purely theoretical concept. It is, in fact, an abbreviation with which to indicate to what extent various arbitrarily chosen valence structures are mixed to describe the bonding in a molecule or a solid. Contrary to Goodman's remark, the formal bond ionicity is defined also for 'dative' bonds.

(3) The covalent bond is defined as arising from the sharing of two electrons between two bonded atoms. It does not specify the charges associated with these atoms and a covalent valence structure cannot, therefore, in general, be taken as the zero of the bond-ionicity scale.

(4) All the definitions given by us[1] and repeated here are in agreement with chemical tradition. The intention of our article was merely to point out that two different meanings are currently attached to the term 'bond ionicity'. To distinguish between them, we introduced the names effective and formal bond ionicities.

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BIOCHEMISTRY

Lipase Activity in the Slow- and Fast-contracting Leg Muscles of the Cockroach

The recent work of Becht and Dresdon[1] and Smit[2] has brought to light certain histological and physiological features of the slow- and fast-contracting mesothoracic leg muscles of the cockroach, *Periplaneta americana*. Smit[2] has shown that the slow-contracting muscle fibres contain more lipids and possess a stronger reducing capacity for methylene blue than the fast-contracting ones. Histochemical investigation of these two types of fibres[3] has shown that there is more lipase activity in the slow-contracting muscles. Here we report the results of a quantitative investigation of lipase activity in the slow- and fast-contracting leg muscles of the cockroach.

Only the metathoracic and mesothoracic leg muscles of the male and female cockroaches were used. According to Becht and Dresdon[1] the muscles 136, 137 and 135*a* and *c* (Carbonell's numbering) are fast-contracting and 135*b*, *d* and *e* are slow-contracting. The slow muscle in this case is the tergo-trochanteral muscle, which is an exceptionally large muscle passing down the coxa to be attached to the trochanter. The fast muscles which are the extensors, and act antagonistically to the above muscle, arise from the coxal rim and are attached on the coxo-trochanteral joint. These two types of muscles were carefully dissected and removed separately from the coxa of the leg into separate ice-cold watch-glasses and weighed. Separate aqueous extracts of the above samples of muscles were prepared at 4° C. The extract was centrifuged at 2,500 r.p.m. for 5 min. and the supernatant was used as the enzyme material in each case. The method used for the assay was adopted from Martin and Peers[4] using a Warburg apparatus. Tributyrin was used as substrate with a bicarbonate–carbon dioxide buffer system of *p*H 7·4 at 37° C. The reaction flask contained 1·5 ml. 0·025 *M* bicarbonate solution and 1 ml. enzyme solution in the main chamber and 0·5 ml. substrate (4 per cent v/v tributyrin in 0·0148 *M* bicarbonate solution emulsified by shaking with a drop of 'Tween 80') in the side arm. The flask and manometer were gassed for 3 min. with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. After equilibrating the flask in the constant temperature water-bath of the apparatus for 10 min., the substrate was tipped and the initial readings were taken after 3 min. The subsequent readings were taken after a 30-min. interval for 1 hr. Lipase activity was calculated on the basis of the protein content of the enzyme solution used and is expressed as μl. carbon dioxide / mgm. protein / hr. Protein was estimated by the micro-Kjeldahl steam-distillation method[5].

Table 1. Lipase Activity in the Slow- and Fast-Contracting Leg Muscles of the Cockroach

Tissue	Lipase activity* (μl. CO_2/mgm. protein/hr.) Male	Female
Slow muscle	129·31	81·43
Fast muscle	75·65	68·47

*Average of 12 experiments.

From the results obtained it is evident that the slow-contracting muscle has a higher lipase activity than the fast-contracting muscle. It is also seen that the activity of the enzyme in the same muscle is considerably lower in the case of the female than that in the male. Again the difference between the concentrations of the enzyme in the two types of muscle is more in the male than in the female. A histochemical study[3] of lipase and certain oxidative enzymes in these muscles also indicated a correspondingly sharp difference in the amount of these enzymes present in these two types of muscles, the slow muscles having more of them. The greater concentration of the enzyme, lipase, in the slow- and fast-contracting muscles could be well correlated with the higher lipid content of these muscles, the slow muscle fibres being more pigmented and showing considerably greater ability to stain for fat than the fast muscle fibres. The higher concentration of lipase and the greater fat content of the slow muscle, which could indulge in sustained contractions for considerably longer periods than that of the fast one, is suggestive of more fat being split for utilization for energy in the former. The higher lipase value in the muscles of the male could also be regarded as an index of the greater capacity for sustained activity involving greater fat utilization in the male.

One of us (N. M. G. B.) is indebted to the Ministry of Education, Government of India, for the award of a senior research scholarship.

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Comparison of the Serum Protein Fractions of the Developing Chicken Embryo by the Technique of Starch-Gel Electrophoresis

Several workers have investigated the serum protein composition of the developing chicken embryo, by moving boundary[1–3] and paper electrophoresis[4,5]. The ultracentrifugal analysis of the serum proteins of the early stage of embryonic development of chicken, as investigated by two groups of workers, are not in agreement in regard to the complexity of the molecular structure in that stage[1,2]. Electrophoretic investigation does show, however, the multiplicity of the components in the early stage, although no complete agreement exists in regard to the number of the components and their relationship to those found in newly hatched and adult birds[6]. Thus Hradec and Lemez[4] reported that the serum proteins of chicken embryo undergo considerable qualitative transformation throughout the development. The technique of starch-gel electrophoresis suggested by Smithies[7] enables one to detect in the serum pattern fractions that cannot be observed by other methods of electrophoresis. The pooled sera of five different age-groups of chicken embryo, incubated for 10, 13, 16, 19, and 21 days, were examined by the technique of starch-gel electrophoresis, run simultaneously on the same gel. The electrophoresis was performed on a vertical tray[8] for 18 hr., using borate buffer of *p*H 8·6 and a field-strength of 4 V./cm. Twelve fractions could be distinguished on the pattern of the serum from the embryo aged 21 days, as is shown in Fig. 1 (two pre-albumins are not shown), as was also indicated in ref. 6. At least nine components (except the pre-

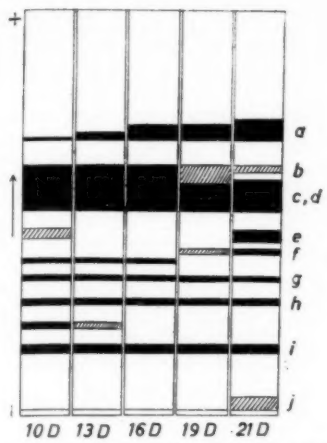

Fig. 1. A schematic representation of the starch-gel electrophoretic pattern of serum of the developing chicken embryo. The letters *a* to *j* refer to albumin and γ-globulin. The numbers 10, 13, 16, 19 and 21 indicate the days of incubation. The striped bands represent the areas that could scarcely be distinguished on the original patterns

albumins) could be distinguished on the pattern from the 10-day old chicken embryo, of which at least seven components corresponded in mobility to those found in the day-old chick. The patterns from the sera of 13, 16, and 19 days of incubation also showed similarity in pattern to that of the day-old chick. A component present in the sera of the 10-day and 13-day old embryo and situated somewhere between components *h* and *j* of 21-day old embryo was not distinguishable in the sera of the older embryos. The results presented here indicate that apparently with the exception of γ-globulins and some other fractions, as demonstrated in Fig. 1, almost all other components present in the day-old chick are present in the serum of 10-day old embryo, as is revealed by the method of starch-gel electrophoresis, and that the transformation occurring throughout this period of development is apparently of a quantitative rather than qualitative nature.

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A Zinc-requiring Alkaline Phosphatase in the Ventral Lobe of the Rat Prostate

Zinc has been shown to play an important part in the activity of phosphatases[1–7]. We have now found evidence for the existence of a zinc-dependent alkaline phosphatase in the ventral lobe of the rat prostate. The hydrolysis of 0·002 *M* adenosine monophosphate in 0·065 *M* *tris*-(hydroxymethyl)-aminomethane buffer at *p*H 9·4 by homogenates of the gland

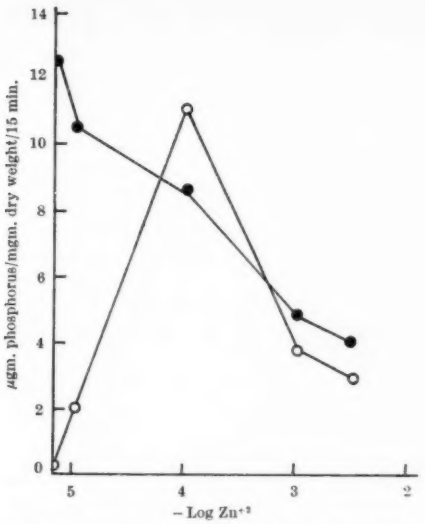

Fig. 1. Effect of zinc on the hydrolysis of adenosine monophosphate by prostatic homogenates from the rat ventral lobe. The effect of the addition of zinc to a homogenate dialysed for 3 hr. against *tris* buffer at *p*H 7·0 at 0° (●) and the addition of 10^{-3} *M* EDTA to the homogenate followed by dialysis for 3 hr. (○). Phosphatase activity was measured by the method of Fiske and Subbarow (ref. 9). Incubation mixture contained 0·5 ml. 0·25 *M* *tris* buffer at *p*H 9·4, 0·1 ml. 0·04 *M* adenosine monophosphate, 0·9 ml. water, 0·5 ml. homogenate; the mixture was incubated for 15 min. at 37°

was progressively inhibited by increasing concentrations of zinc (Fig. 1).

The addition of ethylenediamine tetraacetic acid (EDTA) at concentrations greater than 10^{-3} *M* to homogenates inhibited the phosphatase activity by 30–50 per cent. When EDTA treatment was followed by dialysis against *tris* buffer at *p*H 7·4, the inhibition was practically 100 per cent.

The addition of zinc acetate restored the activity of the inhibited enzyme to about 80 per cent of its original value. Sodium acetate had no effect. After reactivation of the enzyme, increasing the concentration of zinc caused normal inhibition (Fig. 1).

In contrast to zinc ions, magnesium and calcium ions reversed to a slight extent the inhibition caused by EDTA and dialysis. Since the logarithms of the stability constants for EDTA complexes of zinc, calcium and magnesium are in the ratio 16·3 : 10·6 : 8·7 (ref. 8), the greater activity of zinc did not depend on the binding capacity of EDTA.

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Demethylation of the Plant Œstrogen Biochanin *A* in the Rat

Genistein and biochanin *A*, 4′-methylgenistein, have been isolated from red clover and have been found to account for the œstrogenic activity of this plant[1]. Though their œstrogenic potency is very weak, the substances may nevertheless be of importance in animal physiology because they are ingested in considerable amounts by grazing animals.

Very little is known about the metabolic transformations of these compounds. Biochanin *A* was found to be demethylated to genistein by the influence of rumen micro-organisms[2]. The present investigation has been undertaken with the aim of studying the metabolism of biochanin *A* in rats. The compound was synthesized by known methods[3] and labelled with tritium[4].

Each of twenty rats was injected intraperitoneally with 25 mgm. of a fine suspension of biochanin *A* in saline. Urine and fæces were collected separately. An ether extract of the urine was prepared and fractionated on a 'ChroMax' preparative paper column (LKB, Stockholm) with butanol/2 *N* ammonia (1 : 1) as eluant. Radioactive biochanin *A* (12·7 mgm.) and genistein (3·5 mgm.) were isolated from the eluates. Their melting points and ultra-violet and infra-red absorption curves coincided with corresponding values of authentic test samples. Both these isoflavones were isolated in the same way from an ether extract of fæces.

The demethylation of biochanin *A* to genistein was demonstrated by incubation experiments with rat liver slices. The experiments were performed as follows: liver slices, 1–1·2 gm. dry weight, were prepared by hand and incubated with 6 μmoles of tritium-labelled biochanin *A* (specific activity 2 μc. per μmole) in 20 ml. *p*H 7·4 Krebs–Ringer phosphate buffer. The incubation was performed at 37° C. for 2 hr. To the volume of the incubation medium was added 4 times this volume of alcohol. The mixture was centrifugated. The slices were homogenized and extracted with 80 per cent alcohol. The solvent was evaporated in a partial vacuum under nitrogen. The dry residues were dissolved in 5 per cent sodium bicarbonate solution and extracted with ether. The sodium bicarbonate solution contained conjugates of the isoflavones. The ether extracts were evaporated and subjected to paper chromatographic analysis on Whatman No. 3 paper, using benzene/acetic acid/water (165 : 34 : 1) as the solvent system. The isoflavones were located by their absorption in ultra-violet light. The chromatograms were cut into strips, eluted with 80 per cent alcohol, and the eluates analysed for radioactivity in a liquid scintillation counter Ekco *N* 612 at a temperature of −20° C. (Fig. 1 *A*). The radioactive eluates from the genistein spots on the chromatograms were rechromatographed in different solvent systems[2]. They were found to coincide with genistein in all the systems. Incubation experiments were also performed as above with intestine contents and labelled biochanin *A*. No radioactivity except from the biochanin *A* spots could be found on these chromatograms (Fig. 1 *B*). Traces of the radioactivity remained at the starting point on the chromatograms both with material from incubation experiments and controls, probably due to insufficient elution.

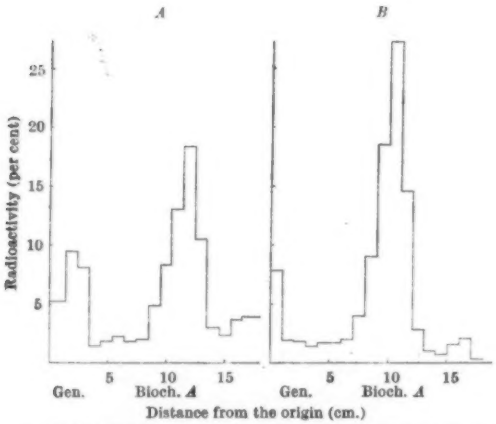

Fig. 1. The distribution of radioactivity on paper chromatograms with biochanin *A*, incubated with liver slices (*A*) and intestine contents (*B*). The radioactivity of the eluates is expressed as percentages of the sum of recovered activity from the chromatogram and indicated on the abscissa. The positions of the test substances are indicated below the diagrams

These preliminary experiments show that a demethylation process occurs in the liver. Several other aromatic ethers have been found to be split in the liver[5]. It is known that methylation of the hydroxyl groups of many synthetic œstrogens retards or decreases their œstrogenic action[6]. It is, therefore, generally believed that these molecules have to be demethylated before becoming active. It is not known if the presence of free —OH groups is also necessary for the action of the isoflavones. However, genistein with three free —OH groups is the most active one of the four œstrogenic isoflavones[7].

Work on the demethylation of methyl ethers of other œstrogens and with different experimental animals is now in progress.

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Enhancement of Steroidogenesis by Rat Adrenal Slices *in vitro* with Œstradiol-17-β

Recent experiments have shown that the concentration of corticosterone in adrenal vein blood of female rats is 2·4 times that in male rats[1]. The possibility that this difference is due, at least in part, to the direct action of œstrogen on the adrenal cortex is the subject of the present study.

Male or female rats of the Sherman strain, weighing 120–150 gm., were maintained in a constant-temperature room for at least one week prior to use. Female rats were bilaterally oophorectomized at 21–30 days of age and used about 6 weeks later. Adrenal slices were prepared according to a modification[2] of the technique of Saffran and Schally[3]. The final incubation medium was modified by addition of U.S.P. adrenocorticotrophic hormone (5 milliunits/100 mgm. adrenal tissue) and œstradiol-17-β $7{\cdot}5 \times 10^{-6}$ *M*. In each experiment, comparable tissue pools were treated by addition of the appropriate diluent alone (saline and propylene glycol respect-

ively). Corticoid yields were determined by ultraviolet absorption and expressed as μgm./100 mgm. adrenal tissue.

The effects of œstradiol addition are shown in Table 1. Significant increments in corticoid production were obtained on addition of œstradiol to male adrenal slices in the absence or presence of adrenocorticotrophic hormone. No effects were observed with female adrenal slices. However, increased corticoid yields, comparable with those obtained with male tissue, were found when adrenal tissue from oophorectomized female rats was employed.

Table 1. EFFECTS OF ŒSTRADIOL ON STEROIDOGENESIS *in vitro*

Adrenal source	No. of observations	ACTH (m.u.)	Corticoid yields* (μgm./100 mgm.) Control	Œstradiol
Male	4	0	9·2 ± 0·6	16·4 ± 1·1†
	4	5	14·9 ± 1·7	20·3 ± 1·8†
Female	4	0	19·1 ± 1·6	20·1 ± 2·0
	4	5	24·8 ± 1·4	25·9 ± 1·0
Oophorectomized female	4	0	11·8 ± 0·8	17·8 ± 0·5†
	4	5	15·5 ± 0·9	19·8 ± 0·9†

* Mean ± standard error.
† Significant at the 1 per cent level.

Administration of adrenocorticotrophic hormone or a standardized stress to adult female rats has been shown to result in significantly higher peripheral plasma corticosterone concentrations than those obtained in male animals[1]. Yates *et al.*[4] have demonstrated that the capacity of liver homogenates from female rats to reduce the *A* ring of corticosterone is significantly greater than that of male animals. These authors have proposed that this more rapid metabolism of steroid is the principal mechanism responsible for greater adrenal secretory capacity in the female rat. The present findings indicate that œstrogen may also affect steroidogenesis directly and independently of its action on liver metabolism. A comparable degree of stimulation was found when œstradiol was added to either male or oophorectomized adrenal tissue. The failure to demonstrate a stimulatory effect on adrenal slices from intact female animals suggests that such tissue has already been subjected to maximal œstrogenic stimulation. The significantly higher control corticoid levels obtained with intact female adrenal tissue compared to tissue from the other sources are consistent with this hypothesis.

Utilizing an essentially similar assay system, McKerns[5] found that the addition of œstradiol-17-sodium sulphate *in vitro* to intact male or female adrenal tissue resulted in no change in corticoid production. The discrepancy between his findings and those of the present work perhaps may be explained by differences in either the form or the dose of œstrogen added.

These results will be discussed in greater detail in a subsequent publication. This study was supported by a research grant (*A*-3370) from the U.S. Public Health Service.

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Female Rat Preputial Glands as a Source of β-Glucuronidase

THE preputial gland of the adult female rat has been shown by Beyler and Szego[1] to be an extremely rich source of β-glucuronidase. However, it may not generally be realized that laboratories needing a steady supply of this enzyme and having access to a rat colony have a cheap and readily available source. Adult female rats which are being discarded can be used, and the two glands, each weighing approximately 50 mgm., are easily dissected free from the surrounding tissue (Fig. 1).

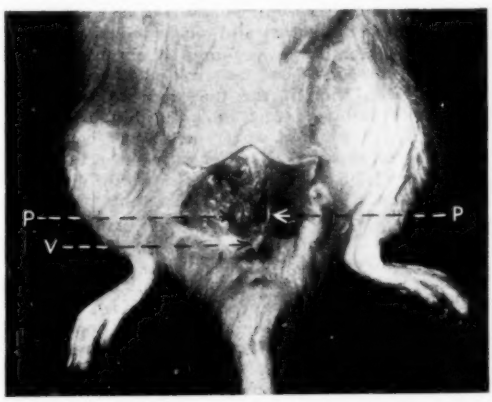

Fig. 1. Adult female rat dissected to show preputial glands. *P*, Preputial gland; *V*, vagina

Homogenization and extraction are then carried out as described by Levvy, McAllan and Marsh[2], though only one ammonium sulphate precipitation is necessary. An equal volume of saturated aqueous ammonium sulphate solution is added to the supernatant obtained, at the end of stage 2 (ref. 2). The ensuing precipitate, after separation from the aqueous phase, is then dissolved in a suitable quantity of 0·1 *M* sodium acetate buffer, *p*H 5·2, so that the resulting solution contains 0·5–1 × 10^6 Fishman units of β-glucuronidase per ml. The enzymic activity of the preparation is assayed by the method of Talalay, Fishman and Huggins[3]. The enzyme solution is stored in the deep freeze and deteriorates only slowly. It does not contain phenolsulphatase, as determined by the method of Roy[4].

The total working time involved in the preparation and assay of one batch is about 2 hr.

Extracts of β-glucuronidase, prepared in this manner from adult female rat preputial glands, have been used for more than four years in the enzymic hydrolysis of steroid glucuronides, notably those of pregnanediol and pregnanetriol.

I am indebted to Mr. A. J. Bailey, of Chelsea Hospital for Women, for the photograph.

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Use of Phenol in determining Chromatographic Purity

Water-saturated phenol is a well-known solvent for the paper chromatographic separation of polyphenols and was, for example, one of the solvents used by Roberts and Wood[1] for the two-dimensional paper chromatographic separation of tea-leaf polyphenols. This solvent was, however, later discarded in favour of water[2], or preferably 2 per cent acetic acid[3].

The use of 75 per cent (w/v) aqueous phenol (or *m*-cresol saturated with water) has proved to be of inestimable value in determining the chromatographic purity of various flavonoid compounds isolated in this laboratory. A fraction isolated from rooibos tea (*Aspalathus acuminatus*) and suspected of consisting solely of isoquercitrin was found by chromatography in phenol to consist of two major flavonoid components even though the material was chromatographically homogeneous in *n*-butanol/acetic acid/water (4:1:2·2 v/v), in water, in 15 per cent acetic acid, in ethyl acetate saturated with water, in propan-2-ol/water (1:3·5 v/v), and in chloroform/butan-2-ol/water (2:4:4 v/v). Separation also took place in 60 per cent acetic acid but was incomplete and not very convincing. The identity of the second component is still under investigation.

In the isolation of quercetin-4′-monoglucoside from the inner scales of the onion (*Allium cepa* L.)[4], difficulty was found in reproducing the melting point of the crystalline material obtained on successive recrystallizations from various solvents. The material was chromatographically homogeneous in all the more commonly employed solvent systems, only phenol and *m*-cresol resolving it completely into two components. The material was therefore purified by chromatography on Whatman 3*MM* paper sheets with 75 per cent (w/v) aqueous phenol as the developing solvent. The contaminant, which had a higher R_F in phenol than quercetin-4′-monoglucoside, crystallized from aqueous ethanol as fine yellow needles with melting point 259–261°.

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Trypsin Inhibitor in Wholewheat Flour

Preliminary work on a trypsin inhibitor in wholewheat flour was reported by Shyamala[1]. A crude concentrate containing the trypsin inhibitor was prepared by the 'ammonium sulphate' method of Borchers *et al.*[2] modified by Lyman[3] as follows:

(1) A homogenate of the wholewheat flour was prepared by mixing the flour with ten volumes of water in a blender. The *p*H was adjusted to 4·2 with acid and the mixture was allowed to stand overnight.

(2) The 'ammonium sulphate precipitate' was obtained by adding ammonium sulphate, 30 per cent of the volume of the extract. The precipitate containing the inhibitor was re-precipitated with ammonium sulphate. The material obtained was dialysed overnight and then lyophilized. Approximately 3 gm. of material was obtained from 300 gm. of flour.

The activity of trypsin was determined by the method of Anson[4] using the modification of Orringer *et al.*[5]. The degree of colour produced by the phenol reagent indicated the relative proteolytic activity of the trypsin. For testing the activity of the inhibitor, it was added to the enzyme at several concentrations and the degree of proteolytic activity inhibited was estimated. A logarithmic plot of this inhibition gave a straight line from which the point of 50 per cent inhibition could be readily obtained.

Crude material (6·5 mgm.) prepared as described here was needed for 50 per cent inhibition of the activity of trypsin while 55γ of a crude concentrate of soybean trypsin inhibitor prepared by the same method was needed for 50 per cent inhibition. The activity was lost when the preparation was heated in a boiling water bath for 20 min.

Table 1. Trypsin-inhibiting Activity of the Inhibitors from Wholewheat Flour and Soybean

Ammonium sulphate precipitation				Trichloroacetic acid	
Soybean inhibitor (γ)	Optical density at 765μ	Wholewheat inhibitor (mgm.)	Optical density at 765μ	Wholewheat inhibitor (mgm.)	Optical density at 765μ
0	0·480	0	0·470	0	0·480
13	0·412	1·0	0·440	1·0	0·395
26	0·355	2·5	0·375	2·0	0·320
52	0·270	5·0	0·285	3·0	0·270
78	0·180	10·0	0·170	5·0	0·172
50 per cent inhibition at 55γ.		50 per cent inhibition at 6·5 mgm.		50 per cent inhibition at 3·5 mgm.	

The crude concentrate prepared as described was used as the starting material for the extraction of the inhibitor with trichloroacetic acid by the method of Kalser and Grossman[6]. The inhibitor appeared in the trichloroacetic acid filtrate while the precipitate had no inhibitor in detectable amounts. The trichloroacetic acid filtrate was dialysed and lyophilized. 3·5 mgm. of this material was needed for 50 per cent inhibition. The appearance of the inhibitor in the trichloroacetic acid filtrate indicates a possible similarity in its behaviour to the pancreatic juice trypsin inhibitor. Further attempts to purify the material are under way.

Recently, Learmonth and Wood[7] reported the presence of a trypsin inhibitor in wheat flour using different assaying techniques.

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PHYSIOLOGY

Prevention of Freezing Damage to Living Cells by Pyridine *N*-Oxide

Pyridine, like dimethyl sulphide, forms a high-boiling, water-soluble oxide, pyridine *N*-oxide (PNO). It was surmised that PNO might protect living cells against freezing damage in the same way as dimethyl sulphoxide[1] and some other aliphatic compounds[2]. The particular interest attached to PNO is that it would be the first aromatic compound to be used in this connexion.

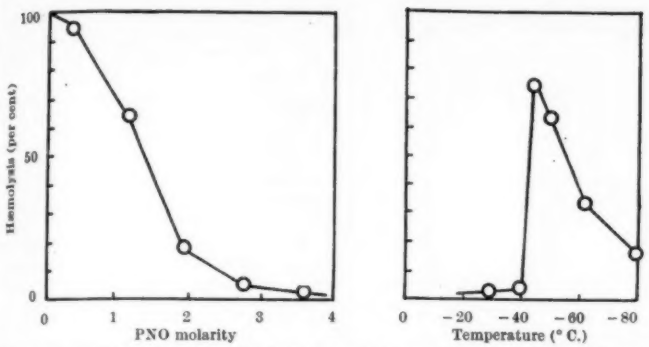

Fig. 1. Hæmolysis of red cells suspended in a solution of pyridine *N*-oxide (PNO) and frozen for 15 min.; as a function of PNO concentration at −40° (left), as a function of temperature with 3·2 *M* PNO (right)

Washed human red blood cells were suspended in buffered saline containing different amounts of PNO and frozen for 15 min. at various temperatures between − 20° and − 80° C. They were then thawed and spun down, and the amount of hæmolysis estimated colorimetrically in the supernatant. Some of the results are shown in Fig. 1, hæmolysis being expressed as a percentage of that found in controls without PNO, at each temperature.

The sharp rise in hæmolysis at − 40°, and slower fall, recalls the behaviour of acetamide at − 51°, and is probably due to a rise in solute concentration to a toxic level[2]. The protective effect is known to depend on permeation of the cells by the solute, and possibly more important than the limited protection found is the fact that PNO is both penetrating and physically non-toxic up to high concentrations. On intraperitoneal injection into mice, PNO had a delayed toxicity (LD_{50} 1·5 gm./kgm.) rather less than that of pyridine[3], to which it is probably reduced *in vivo* (could be smelt in the viscera). The high solubility of PNO in water is believed to be due to localization and enhancement of the three lone-pairs of electrons on the oxygen, by mesomerism with the aromatic ring. It is hoped to discuss the structure of such highly soluble compounds in a later communication.

I am indebted to Mr. M. J. Ashwood-Smith for carrying out the toxicity experiments.

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Influence of Anaerobic Glycolysis on Release of Histamine in the Guinea Pig and Rat Anaphylactic Reaction *in vitro*

An increase of oxygen uptake has been observed during the anaphylactic reaction *in vitro*[1], and it has been shown that anaphylactic release of histamine is inhibited by lack of oxygen[2–4]. It has also been shown that the anaphylactic reaction is inhibited by uncouplers of the oxidative phosphorylation[5]. It would be expected that the energy-rich compounds formed during the glycolytic cycle would be available for release of histamine, as are those generated in aerobiosis. The present investigation was undertaken in order to see if the anaphylactic reaction depends on oxidative metabolism, or if it could also proceed in the presence of an anaerobic metabolism. The experiments were carried out with both guinea pig and rat tissues, in order to verify any possible difference in their behaviour, since it has recently been suggested that anaphylactic reaction in rat tissues is not affected by lack of oxygen[6,7].

Sensitized guinea pig and rat lung-slices were incubated in Ringer-phosphate or Ringer-bicarbonate medium with or without glucose, in nitrogen or oxygen atmosphere, in Warburg flasks in which release of histamine was determined by the addition of antigen from the side arm. Nitrogen was purified by passing it through melted phosphorus, and its purity verified in an Orsat apparatus[4]. Rats were sensitized with horse serum treated by kaolin, according to the method in ref. 8. The technique of histamine dosage and other experimental details were as described in ref. 5.

It has already been shown that a pure nitrogen atmosphere completely inhibits anaphylactic release of histamine in guinea pig lung-slices[4]. In the presence of glucose the sensitized guinea pig lung-slices, maintained in anaerobic conditions, showed as would be expected a glycolytic activity, while histamine was released on tipping the antigen. Addition to the glucose anaerobic medium of mono-iodoacetate, well known as an inhibitor of the anaerobic glycolysis, completely inhibited this release. Table 1 shows the results obtained in typical experiments.

Table 1. Effect of Glucose and Mono-iodoacetate on Metabolism and on Antigen Histamine Release from Sensitized Guinea Pig Lung-Slices under Anaerobic Conditions

Experimental conditions		Metabolism		
Atmosphere	Additions	Oxygen uptake Q_{O_2}	Glycolysis Q_{CO_2}	Histamine release (μgm./gm. w.w.)
Nitrogen + 5 per cent carbon dioxide	—		0·85 ± 0·09	0·0 ± 0·1
Nitrogen + 5 per cent carbon dioxide	Glucose		5·69 ± 0·34	3·2 ± 0·7
Oxygen	—	5·44 ± 0·30		3·9 ± 0·3
Oxygen	Glucose	5·25 ± 0·36		4·9 ± 0·3
Nitrogen + 5 per cent carbon dioxide	Glucose		6·90 ± 0·54	10·7 ± 0·6
Oxygen	—	5·90 ± 0·40		4·1 ± 0·1
Oxygen	Glucose	6·00 ± 0·19		4·7 ± 0·5
Nitrogen	Glucose	0·11 ± 0·10		5·3 ± 0·5
Nitrogen + 5 per cent carbon dioxide	Glucose		7·98 ± 0·45	17·5 ± 0·4
Nitrogen + 5 per cent carbon dioxide	Glucose + monoiodo-acetate, 1 m*M*		0·45 ± 0·10	0·0 ± 0·1
Nitrogen + 5 per cent carbon dioxide	Glucose		7·06 ± 0·27	11·3 ± 0·4
Nitrogen + 5 per cent carbon dioxide	Glucose + monoiodo-acetate, 0·5 m*M*		0·73 ± 0·09	0·4 ± 0·3

Medium was Ringer-phosphate, or Ringer-bicarbonate when atmosphere contained carbon dioxide. Glucose, 0·01 *M*. Spontaneous release of histamine was calculated from these data. Each figure is the mean of results from three flasks ± *S.E.*

In sensitized rat lung-slices, anaerobiosis completely inhibited the histamine released by antigen and small quantities of oxygen were sufficient to remove this inhibition. Addition of glucose to the medium also removed the anaerobiosis inhibition (Table 2).

Table 2. Effect of Total and Partial Anaerobiosis and of Glucose in Anaerobic Conditions on Metabolism and on Antigen Histamine Release from Sensitized Rat Lung-Slices

Experimental conditions Atmosphere	Additions	Metabolism Oxygen uptake Q_{O_2}	Glycolysis Q_{CO_2}	Histamine release (μgm/gm. w.w.)
Oxygen	—	7·23 ± 0·35		2·9 ± 0·3
Nitrogen	—	0·65 ± 0·05		0·0 ± 0·0
Oxygen	—	6·34 ± 0·11		2·3 ± 0·3
Nitrogen	—	0·70 ± 0·14		0·0 ± 0·0
Oxygen	—	7·75 ± 0·12		1·4 ± 0·15
Nitrogen + 2 per cent oxygen	—	1·58 ± 0·07		1·0 ± 0·1
Oxygen	—	8·10 ± 0·20		3·9 ± 0·2
Nitrogen + 4 per cent oxygen	—	2·15 ± 0·15		3·6 ± 0·3
Oxygen	—	7·30 ± 0·14		3·5 ± 0·3
Nitrogen + 5 per cent carbon dioxide	Glucose		6·52 ± 0·14	3·5 ± 0·0

Same experimental conditions as in Table 1. Each figure is the mean of results from two flasks ± *S.E.*

These experiments show that in both guinea pig and rat-sensitized lung-slices the anaphylactic histamine release is not specifically dependent on oxygen. This reaction is able to proceed in complete anaerobiosis, if energy is available to the cell, such as that produced by a carbohydrate glycolytic metabolism.

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Effect of Monovalent Cations on the Blood Pressure of Intact and Hypertensive Rats

We demonstrated in earlier experiments that it was possible to inhibit renal neurogenic hypertension in rats when potassium salts were mixed with their food[1]. It was likewise demonstrated that the degree of Grollman hypertension did not depend on the absolute quantity of sodium and potassium in the food but on the mutual proportions of the two electrolytes[2]. Meneely's experiments[3] gave similar results: rats, made hypertensive by excessive doses of sodium chloride, could be kept alive and the rise of their blood pressure stopped by feeding them potassium salts. The blood pressure of hypertensive patients can be lowered by a high potassium diet[4].

Excessive consumption of sodium chloride is known to promote hypertension in human beings and animals alike. Many workers ascribe this to the higher hydration of the sodium ion which leads to a swelling of the arterial walls[5]. According to Pallman[6], an atom of sodium binds 4·3, and an atom of potassium only 0·9 mol. of water. If the hypertensive effect of sodium and the anti-hypertensive effect of potassium depend on hydration, it would be justified to expect that lithium, as the most hydrated monovalent cation, would produce higher blood pressure than sodium, and that rubidium and cæsium, which are less hydrated, would produce a stronger depressing effect than potassium. (An atom of lithium binds 10, that of rubidium 0·5 and cæsium 0·2 mol. of water.)

In order to test this hypothesis we mixed rubidium and cæsium salts (Rb_2SO_4; CsCl) to the diet of male rats of 150–180 gm. body-weight which had been made hypertensive by Grollman's method. We also administered lithium salts (LiCl) to such animals since they refused the food which contained lithium if it was given by means of intraperitoneal injection. The daily doses of rubidium and cæsium were 15 mgm., those of lithium 4 mgm. The basal diet contained 26 mgm. of sodium and 61 mgm. of potassium per day, while there was 120 mgm. of potassium in the 'high-potassium diet'. Figs. 1 and 2 illustrate changes in the blood pressure of intact and Grollman-hypertensive animals after administration of different monovalent cations. We determined the blood pressure with our method[7] once a week. It can be seen from Fig. 1 that the blood pressure of the intact lithium-fed animals already showed an increase during the second week, while the blood pressure measured in rats which had been kept on a high-potassium diet or received rubidium or cæsium did not differ from that of animals kept on a normal diet.

By keeping rats, operated by Grollman's method, on a high-potassium diet or by administering rubidium or cæsium to them, it was possible to arrest a further increase in their blood pressure (Fig. 2). Cæsium inhibited the development of hypertension almost completely: after a small increase during the first weeks, blood pressure went back in the sixth week to the pre-operative (normal) level.

Our experiments allow the conclusion that all monovalent cations are capable of influencing the blood pressure of rats. Effect on blood pressure depends on the hydration of the atoms. Cations of high hydrating power (lithium, sodium) promote hypertension. This property of sodium ions has long been known. Lithium has now been proved to be a substance which enables us to produce experimental hypertension in an easy and reliable manner. This discovery seems to be of great significance in the field of experimental hypertension since it is now possible to study problems concerning the pathogenesis and treatment of hypertension promptly, without surgical intervention and without the administration of mineralocorticoids (in other words, under compara-

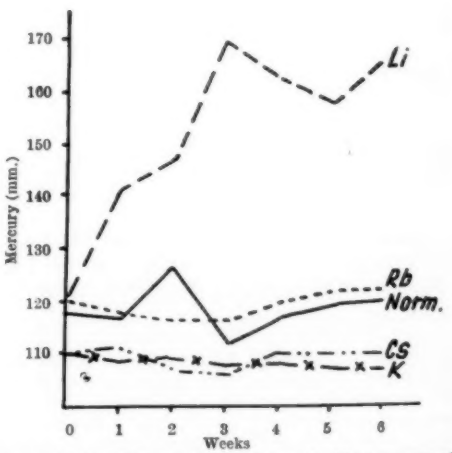

Fig. 1. Action of Li, K, Rb and Cs on the blood pressure of intact rats. Norm., normal diet; Li, normal diet plus 4 mgm. Li; K, normal diet plus 59 mgm. K; Rb, normal diet plus 15 mgm. Rb; Cs, normal diet plus 15 mgm. Cs

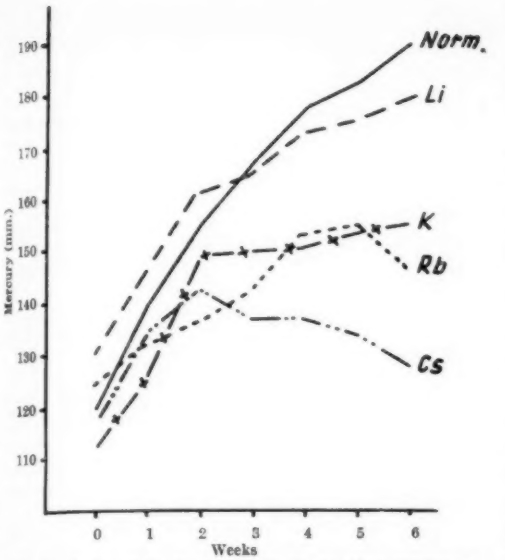

Fig. 2. Action of Li, K, Rb and Cs on the blood pressure of Grollman-operated rats (key, see Fig. 1)

tively more physiological conditions). The prevention of hypertension by means of rubidium and cæsium also supports (but does not definitely prove) our working hypothesis that the action of monovalent cations on blood pressure, as also the direction of the effect, depends on the degree of ionic hydration.

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Ambient Temperature and Depot Fat Iodine Number in Children

The degree of unsaturation of depot fat, as measured by its iodine number, has been shown to depend on species, temperature of the environment, diet and site within the body of the fat[1]. During the course of investigations in Bantu children aged up to 4 yr. in South Africa it was found that as the child got older, so the iodine number of the depot fat (taken from the anterior abdominal wall) increased and the difference between the means of the group under 1 yr. old and those aged 1 yr. was significant ($P = 0{\cdot}025 - 0{\cdot}01$) and between the means of 1 yr. and 3 yr. the significance was 1 in 20. Comparison with the 2-yr.-old group did not show any significance owing to the small number in the group (Fig. 1).

These findings, it is suggested, are consistent with the view that the degree of unsaturation of the depot fat in children is related to the temperature of the environment, as has been shown in animals[2]. Of the factors influencing depot fat iodine number the site within the body and species variability need not be considered here. The composition of the fat in the diet has been shown to alter the composition of adipose tissue, but relatively large quantities of fat have to be consumed in order to demonstrate this effect[3]. These children have no excess of dietary fat, their highest fat intake probably being while breast fed (fat in breast milk = 4 per cent).

Furthermore, the fat consumed by the mother and which is to some extent reflected in the composition of her milk[4] is the same fat as would be consumed by the child when weaned.

The difference with age in the South African series could be explained by differences in temperature of the environment to which the child is exposed. When this was done the atmospheric temperature varied from 5 to 18° C. During early life the child is not exposed to this temperature for long periods of the day as it is carried wrapped in blankets on its mother's back. As it gets older it spends longer intervals of time away from its close contact with the mother and thus the temperature to which the skin is exposed will have lower mean values. The iodine number of the depot fat in adult Bantu in South Africa is 67 (ref. 5).

In a second series of children in West Africa where the atmospheric temperature was 28–33° C. the iodine values showed no rise with age and were similar to those of the 0–1 yr. age group in South Africa (Fig. 1). The atmospheric temperature in West Africa was therefore close to that which would exist when the child is tied to its mother's back. Gradual 'weaning' from the mother's back would not be associated with any marked fall in temperature

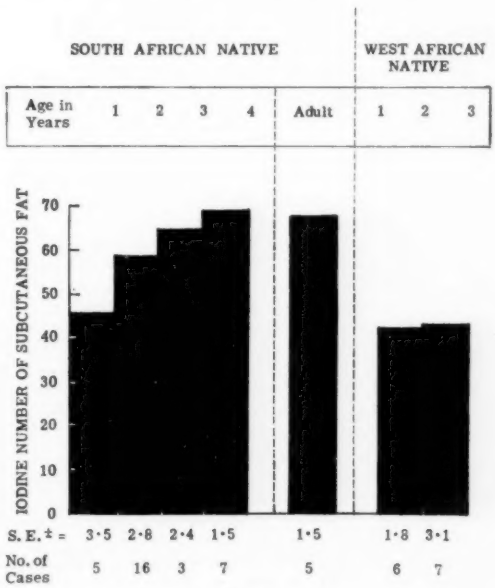

Fig. 1

exposed to the skin, and therefore no striking change in the degree of unsaturation of depot fat, and could account for these older children having the same iodine number as the very young age group in South Africa.

It is therefore concluded that these results in children would be consistent with the hypothesis that the iodine number of depot fat is determined, to some extent, by the ambient temperature.

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Seminal Degeneration induced in Merino Rams by the Administration of Stilbœstrol Dipropionate

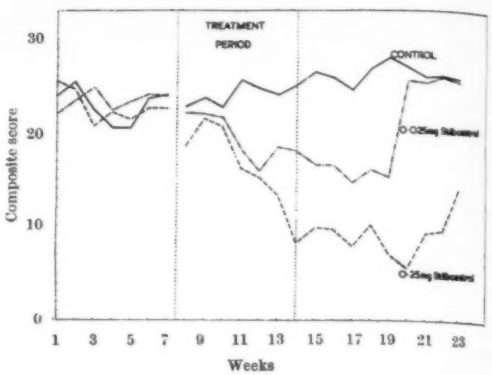
The statement that rams are unaffected by the ingestion of œstrogenic pasture seems to be based on the failure to detect lowered fertility following mating[1], or metaplastic changes in the bulbo-urethral and prostatic glands of rams from properties where ewes exhibit the syndrome of 'clover infertility'[2,3]. Substantiation has been drawn from the protection conferred by the administration of androgens to castrates treated at the same time with stilbœstrol[4]. No reports have been noticed of the results of semen examination of rams fed œstrogenic pastures or treated with synthetic œstrogens, although it has been shown that injections of 35 mgm./week of œstradiol benzoate may alter semen characters of bulls in 3 weeks; these returned to normal within 5 weeks of the cessation of treatment[5].

In experiments conducted at the Ian Clunies Ross Laboratory, fifteen Merino rams, aged approximately 24 months, were divided at random within live weight classes into three groups; mean live weights were 45 kgm.

Semen samples were collected from each ram by electro-ejaculation[6] at weekly intervals for 23 weeks beginning August 17, 1960. The samples were assessed for volume, sperm density and total sperm numbers, motility and for the percentages of morphologically normal and live sperm. After a pre-treatment period of seven weeks, to allow each group to establish reasonably consistent base-lines, treatments were begun. These consisted of intra-muscular injections of stilbœstrol dipropionate at daily dose-rates of 0·025 mgm./sheep for group 1 and 0·25 mgm./sheep for group 2. Peanut oil alone was injected into the control group 3. Treatments continued between the eighth and fourteenth weeks inclusive of the experiment, and were then stopped.

Sperm motility was scored visually between 0 and 10, and the percentages of live and of normal sperm were converted to a scale of 0–10 by taking each 10 per cent as one point. Because of the large variation that occurs in total sperm numbers in semen samples collected by electro-ejaculation (Mattner and Voglmayr, unpublished) this character was disregarded in the composite scores.

Mean total scores for each week for each group of rams are shown in Fig. 1, which reveals a depression in semen quality as the result of the treatments. Group 2, which received 0·25 mgm. stilbœstrol, had not completely recovered within 9 weeks of treatment being stopped, but group 1, which received 0·025 mgm. stilbœstrol, showed substantial recovery within 6 weeks of treatment ceasing. The control group remained unaffected.



Of the individual semen characters examined, motility was most adversely affected, and in group 2 this fell to zero by the seventh week after stilbœstrol injections began. The mean percentage of live sperm in semen samples from this group fell from 85 per cent before treatment to 40 per cent by the seventh week of treatment, and to 20 per cent by the twentieth week; that is, 6 weeks after treatment ceased. The mean total sperm numbers of both groups 1 and 2 began to decline 5 and 7 weeks respectively after treatment commenced. Four weeks after these declines became evident, mean total sperm numbers obtained from group 2 at each collection ranged between 100×10^6 and 300×10^6 and from group 1 between 500×10^6 and 1000×10^6. No upward trend occurred in group 2 until 9 weeks after treatment ceased; mean sperm numbers for group 1 rose to 2000×10^6 within 3 weeks and were maintained at this average until the ninth week after the administration of stilbœstrol stopped. Mean total sperm numbers obtained from the control group during the whole of the experiment fluctuated between 4000×10^6 and 5000×10^6.

Rams grazing highly œstrogenic pastures in Australia are unlikely to ingest the equivalent of 0·25 mgm. stilbœstrol/day administered to group 2. Dose-rates of 0·03 mgm. diethylstilbœstrol/sheep/day for 6 months adversely affected the breeding performance of ewes[7]. Preliminary observations (Southcott, Braden and Moule, unpublished) indicate that Merino ewes grazing red clover (*Trifolium pratense*) pastures at Armidale (New South Wales) may have an œstrogen intake equivalent to 0·015 mgm. stilbœstrol dipropionate/day, but bio-assay of collected subterranean clover (*Trifolium subterraneum*) fed to castrated guinea pigs has shown far higher œstrogenic equivalents[4]. It is clear that the dose-rate of œstradiol benzoate/kgm. live weight required to produce seminal degeneration in bulls[5] could be as much as twenty times the dose of stilbœstrol dipropionate/kgm. live weight required to affect the semen of rams; thus it is unlikely that bulls could consume sufficient œstrogenic pasture to affect spermatogenesis. The evidence available indicates that cattle are unaffected by grazing pastures that severely affect sheep[1].

It should not necessarily be concluded that grazing pasture possessed of high œstrogen equivalencies will induce seminal degeneration in rams. So far the activity of pasture in terms of stilbœstrol dipropionate has been estimated by the use of castrated male sheep and spayed ewes allowed to graze the pasture under test. On assimilation by the entire male, the isoflavones formononetin, genistein and biochanin A, and diadzein and the lactone coumestrol may not lead to the formation of substances likely to impair spermatogenesis. Furthermore, this experiment was conducted at a time of the year when the androgen output by the rams may have been low.

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PHARMACOLOGY

Repetitive After-discharge produced by Drugs in Nerves treated with Cetyltrimethylammonium Bromide

A well-established pharmacological property of tetraethylammonium ion (TEA) is the production of repetitive nerve action potentials in response to single stimuli[1]. We have recently been examining the relationship between quaternary ammonium ion structure and this type of activity. After several experiments, however, it became apparent that the effects of TEA and other ethonium ions were not reproducible except at very high concentrations of 30 m*M* or more. Accordingly, we resorted to testing these drugs on nerves treated with the cationic detergent, cetyltrimethylammonium bromide (CTMB). Certain methonium ions which normally have little or no effect on nerve produce conduction block after CTMB treatment[2,3]. The experiments described here reveal that CTMB also increases the capacity of ethonium ions to produce repetitive firing.

All experiments were done on sciatic nerves isolated from cold-adapted winter and spring frogs (*Rana pipiens*). Except for brief stimulating and recording intervals in air, the nerves were bathed in a buffered frog Ringer solution (*p*H 7·2) at a temperature of 16–18° C. Monophasic action potentials were recorded with differential amplification, displayed on an oscilloscope, and photographed. A square-wave stimulus of 0·1 m.sec. duration was applied every 2 sec. Stimulus intensity was supramaximal for the rapidly conducting *A* fibre group. The nerve chamber contained a small centre well located between the platinum stimulating and recording electrodes. Drugs and CTMB were added to the Ringer solution in this centre well only. The so-called treated segment of the nerve was initially bathed in CTMB–Ringer for 60 min., then in CTMB–Ringer–drug solution. CTMB concentration was kept at 1 μgm./ml.

The compounds used in these experiments were TEA chloride, phenyltriethylammonium iodide and 3-OH phenyltriethylammonium iodide. With the appropriate concentration of each compound, two effects were consistently observed: (1) an increase in amplitude and time-course of the negative after-potential; (2) repetitive after-discharge. Summation of asynchronous and low-amplitude repetitive spikes of many individual axons prevents accurate measurement of the negative after-potential. Therefore, only the repetitive firing was used as an indicator of drug action, and this response was treated as a graded one. The frequency, number and amplitudes of repetitive spikes were concentration and time dependent.

Fig. 1. Repetitive after-discharge produced by 5 μgm./ml. phenyltriethylammonium ion in presence of CTMB. Left, control record taken after 30 min. in Ringer; middle, threshold response obtained after 5 min. in CTMB–Ringer–drug solution; right, maximal response obtained after 30 min. in CTMB–Ringer-drug solution. Time line, 1,000 cycles/sec.

The approximate concentration of each ethonium ion required for a minimal response was determined on at least 4 normal and 4 CTMB-treated nerves. A typical response is illustrated in Fig. 1. For each ion, CTMB markedly lowered the approximate concentration required for threshold response (Table 1). The time between drug application and onset of effect was not altered by CTMB when using threshold concentrations; but when the threshold concentration as determined on a normal nerve was applied to a CTMB-treated nerve, the latent period was too short to measure. Our results might have been even more striking had we used a higher CTMB concentration. However, we wished to avoid the direct actions of CTMB on ion permeability which were recently observed in the giant axon of the squid[4].

Table 1. Comparison of Drug-induced Repetition in Normal and CTMB-treated Nerves

Drug	Normal nerves		CTMB-treated nerves	
	Threshold concentration* (m*M*)	Time to onset† (min.)	Threshold concentration* (m*M*)	Time to onset† (min.)
Tetraethylammonium	10–20	20	0·5–2	45
Phenyltriethylammonium	20–30	30	3–5	30
3-OH phenyltriethylammonium	30–40	10	3–10	13

* Range of approximate threshold concentrations on 4–7 nerves.
† Average of results obtained on 4–7 nerves.

Koketsu's experiments[5] indicated that TEA prolongs the negative after-potential in frog motor nerve terminals. The concentrations used by Koketsu approximated those needed in the present work to produce repetitive firing of CTMB-treated axons. The unmyelinated axon terminals are more sensitive than the rest of the axon to TEA, probably because the latter possesses a permeability barrier which impedes the access of quaternary ammonium ions to their sites of action. It is this barrier which a cationic detergent like CTMB appears to remove.

XUM

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Cholinergic Transmission Mechanisms for both Excitation and Inhibition in Molluscan Central Synapses

We have previously reported[1] that the application of acetylcholine by perfusion or local injection to different central neurones of the mollusc *Aplysia depilans*, impaled with intracellular microelectrodes, always produced one of the two following reactions: (*a*) cells of one type, conventionally designated by us as *H*-neurones, are hyperpolarized and inhibited by the drug (Fig. 1, *H*1), and (*b*) cells of another type, or *D*-neurones, are depolarized and excited (Fig. 1, *D*1).

Further microphysiological analysis revealed that *H*-neurones receive both excitatory and inhibitory inputs, but that *D*-cells receive only excitatory input. A pharmacological investigation enabled us to demonstrate that acetylcholine fulfils almost all the requirements necessary in order that it be accepted as the chemical transmitter of synaptic inhibition in *H*-neurones[1,2].

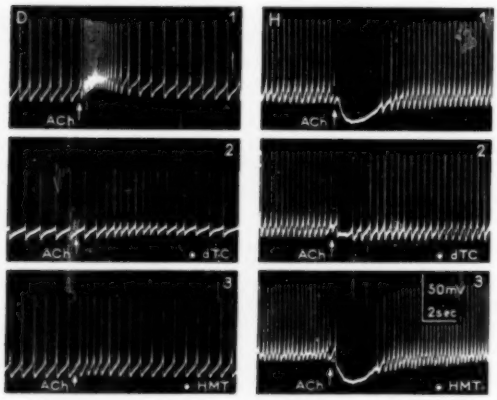

Fig. 1. (1) Effect of brief electrophoretical injections of acetylcholine (arrows) on the somatic membrane of a *D*-cell (left column) and an *H*-cell (right column) firing spontaneously. The drug depolarizes the *D*-cell and accelerates its firing, while it hyperpolarizes the *H*-cell and inhibits firing.
(2) Same amount of acetylcholine as in (1) is injected on the *D*-cell (left) and the *H*-cell (right) but in the presence, in the bathing fluid, of 10^{-4} gm./ml. of *d*-tubocurarine, which clearly inactivates both *D*- and *H*-cell membranes to the action of acetylcholine.
(3) The same electrophoretical injection of acetylcholine on *D*- and *H*-cells, as in (1), but in the presence, in the bathing medium, of 10^{-4} gm./ml. of hexamethonium bromide. This latter drug inactivates the *D*-cell membrane to the action of acetylcholine (left) but has no effect on the response of the *H*-cell membrane to acetylcholine

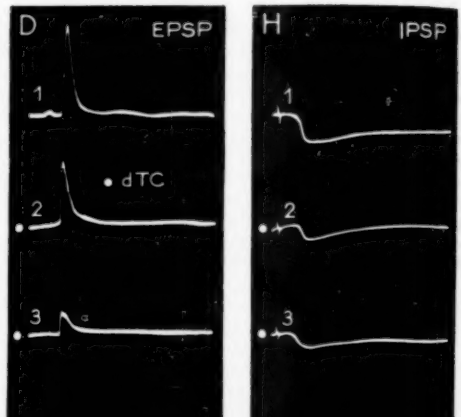

Fig. 2. Effects of *d*-tubocurarine in 10^{-4} gm./ml. concentration (white dots, 2–4) on the evoked excitatory postsynaptic potential in a *D*-cell (left column) and on the inhibitory postsynaptic potentials in an *H*-cell (right column). The synaptic potential in the *D*-cell is composed of two elementary excitatory synaptic potentials. In both cells the drug inactivates the postsynaptic membrane to natural excitatory and inhibitory transmitters respectively, as indicated by the gradual diminution (2–4) of the postsynaptic potentials. The removal of the curare restores the initial amplitude of the synaptic potentials (5)

Recent experiments brought out the surprising result that acetylcholine may also very likely be considered as the chemical transmitter for synaptic excitation in *D*-neurones. These neurones are highly sensitive to the drug; threshold effects on the cell excitability were observed with concentrations of less than 10^{-10} gm./ml. in absence of any anticholinesterase. Applied either by perfusion or locally injected, acetylcholine produces a spectacular drop in the *D*-cell membrane impedance, as in the neuromuscular end-plate of vertebrates[3] and the Purkinje fibres of vertebrate heart[4], where its transmitter action is well proved. Detectable increases of membrane conductance were observed for concentrations of acetylcholine down to 10^{-8} gm./ml. with a square pulse technique permitting the detection of impedance variations as small as 2–3 per cent.

Electrophoretical injections of acetylcholine on the *D*-cell membrane, following the technique of del Castillo and Katz[5], resemble the excitatory synaptic action (Fig. 1, *D*1). Like that of the excitatory synaptic potential, the equilibrium potential-level of this action is closer to the zero potential than the resting potential; if the membrane potential is artificially increased, the amplitude of both excitatory postsynaptic potential and depolarization produced by acetylcholine increases. The effects of electrophoretical injections of acetylcholine on the *D*-cell membrane (as well on the *H*-cell membrane) are diminished or abolished in the presence of a solution 10^{-4} gm./ml. of *d*-tubocurarine (Fig. 1, *D*2 and *H*2) or atropine. This block may probably be attributed to the inactivating effects caused by these drugs on the

cell membrane cholinergic receptors. It is highly significant that, in the presence of *d*-tubocurarine or atropine, the excitatory postsynaptic potentials in *D*-cells (and the inhibitory postsynaptic potentials in *H*-cells) also show rapid diminution of their amplitude or even disappear (Fig. 2, *D* and *H*). This inactivating effect of curare and curare-like drugs on synapses constitutes an important argument in favour of the cholinergic nature of the synapses[6,7].

The action of hexamethonium was very remarkable (Fig. 1, *D*3 and *H*3). In the presence of this drug, the *D*-cell membrane is inactivated to the action of the injected acetylcholine, whereas the *H*-neurone membrane is not affected and shows a normal hyperpolarizing response to acetylcholine. Likewise, hexamethonium greatly diminishes the amplitude of the excitatory postsynaptic potentials in the *D*-neurones, but has no effect on the inhibitory postsynaptic potentials in *H*-cells. This behaviour illustrates once more the pharmacological specificities of the *D*- and *H*-cell membranes, seen also with several other drugs[8].

The foregoing arguments seem to us to be a rather strong indication in favour of the cholinergic nature of the excitatory synaptic input in *D*-cells, especially if we remember that acetylcholine is normally present in *Aplysia* nervous system[9] and that a specific cholinesterase was demonstrated by both biochemical[10] and physiological[2] methods. As in *H*-neurones, the extrasynaptic membrane of the cell body is highly sensitive to the natural transmitter.

Recalling that the synaptic excitatory input of *H*-neurones is not at all affected by drugs which block cholinergic transmission[2], we presume that the excitatory synaptic input is conveyed by an unknown transmitter other than acetylcholine.

Fig. 3 illustrates the nature of the synaptic inputs on *D*- and *H*-cells. As the excitatory synaptic transmission in *D*-cells and the inhibitory one in *H*-cells appear to be cholinergic, the existence of interneurones capable of excitatory action through some of their synaptic endings and inhibitory actions through others at the same time is likely (dotted lines in Fig. 3). This dual cholinergic mechanism emphasizes the importance of the postsynaptic membrane for the character of the transmission, the same transmitter in the same ganglion being able to produce opposite synaptic actions.

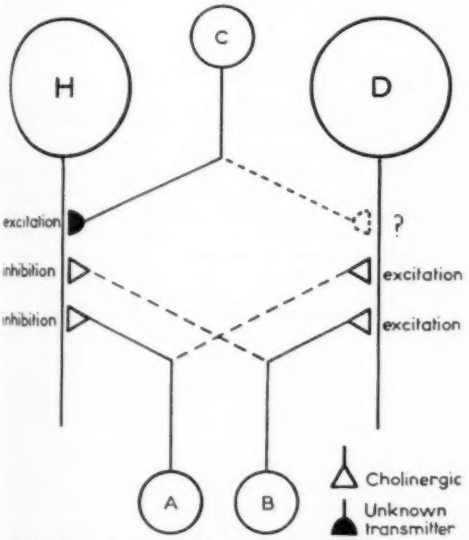

Fig. 3. Diagram illustrating the nature of synaptic contacts on the *H*- and *D*-cells. The inhibitory input to the *H*-neurone and the excitatory one to the *D*-cell are cholinergic. A probable contact of a single presynaptic neurone (either *A* or *B*) with both *H*- and *D*-cells is indicated by the dotted lines. The excitatory input on the *H*-cell is conveyed by an unknown transmitter different from acetylcholine. A possibility of contact between the presynaptic cell *C* and the postsynaptic *D*-neurone is marked with a dotted line; no evidence exists concerning the effect of the unknown transmitter on the *D*-neurone

Conclusions from the above results may be summarized as follows: (1) As was demonstrated elsewhere[1,2], acetylcholine, by its effects on *Aplysia depilans* neurones, enables one to distinguish two types of cells: *D*-cells, depolarized and excited by the drug, and *H*-cells, hyperpolarized and inhibited. The *D*-neurones only show excitatory synaptic input, whereas *H*-neurones present both excitatory and inhibitory synaptic input. (2) In *D*-neurones, the synaptic excitatory input may be considered as cholinergic. (3) In *H*-neurones, inhibitory input is also very likely cholinergic, but the excitatory input in these cells appears to depend on another, unknown chemical transmitter. Hence, the character of synaptic transmission, excitatory in *D*-cells and inhibitory in *H*-cells, does not depend solely on the nature of the chemical transmitter—acetylcholine in both cases—but on physicochemical differences in the postsynaptic membrane that react in a specific way to the transmitter. (4) Existence of interneurones which would simultaneously induce excitatory and inhibitory synaptic actions in different postsynaptic neurones is a real possibility.

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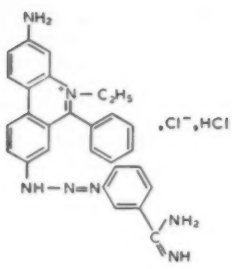
CHEMOTHERAPY

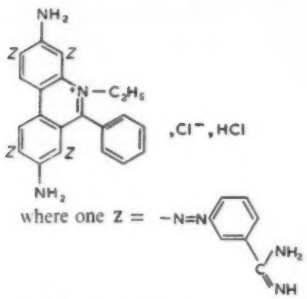
A New Prophylactic Trypanocidal Drug, 2,7-di-(*m*-amidinophenyldiazoamino)-10-ethyl-9-phenyl-phenanthridinium Chloride Dihydrochloride (*M & B* 4596)

The properties of the trypanocidal drugs obtained from the coupling reaction of *m*-amidinobenzenediazonium chloride and homidium chloride have been described by Wragg, Washbourn, Brown and Hill[1], and Berg[2]. Metamidium was the mixture of the red (I) and purple (II) isomers (45 and 55 per cent respectively), and isometamidium was the pure red isomer (I).

In the study of the structure–activity relationships among this series of compounds, many similar coupling reactions have been investigated. In an attempt to prepare the 2,7-di-*m*-amidinophenyl-



(I)



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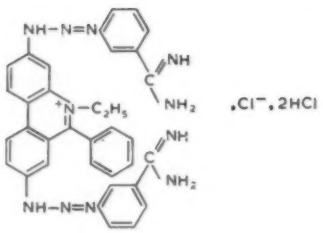

(III)

diazoamino derivative (III) of homidium, the coupling reaction of 2 mol. of *m*-amidinobenzenediazonium chloride and 1 mol. of homidium chloride was examined. Only (I) and (II) were present in the reaction product and none of the desired *bis*-compound (III) was obtained. The latter compound, however, was readily prepared by the coupling reaction of tetrazotized homidium chloride (1 mol.) and *m*-aminobenzamidine monohydrochloride (2 mol.). It separated from the reaction mixture as a brown granular solid, which was washed with aqueous sodium chloride and dried at room temperature. It usually contained approximately 10 per cent of sodium chloride and was submitted for biological examination without further purification as *M & B* 4596. It decomposed at 245°, and was approximately 1·5 per cent w/v soluble in water; the *p*H of a 1 per cent w/v solution being 4·7 at 20°. It could be stored in the solid state for at least two years without significant decomposition, but it began to lose nitrogen when heated above 70°. Aqueous solutions lost nitrogen when warmed above 40°. On heating (III) with cuprous chloride and 3 *N* hydrochloric acid for 7–10 min. the theoretical amount of nitrogen was evolved.

Table 1. Toxicity and Therapeutic Activity against a Laboratory Strain of *T. congolense* in Mice

Compound	*LD*50 (mgm./kgm.) subcutaneous	*CD*50 (mgm./kgm.) by subcutaneous injection
Homidium	75	0·03
Metamidium	210	0·0075
Isometamidium	260	0·002
M & B 4596	> 1,000	0·6

Table 2. Prophylactic Activity of *M & B* 4596 against a Laboratory Strain of *T. congolense* in Direct Comparison with Metamidium Chloride

Compound	Dose (mgm./kgm.) subcutaneously	No. of mice protected/No. treated, Weeks after dosing: 2	3	4	6	9	12	16	20	24
Metamidium chloride	25		6/6		5/5	6/6	11/11	10/10	11/14	8/10
	5			4/4		4/6				
	1	6/6		19/19	9/14					
M & B 4596	25		6/6		5/5	6/6	20/20	19/21	15/15	10/10
	5			4/5		4/4				
	1	5/6		11/19	9/16					

The therapeutic and prophylactic activity of the compound against a laboratory strain of *Trypanosoma congolense* was determined by the methods described by Wragg *et al.*[1], and the results are summarized in Tables 1 and 2. The compounds were all examined as their chloride hydrochlorides, and the doses are given as weight of total salt.

Although *M & B* 4596 was much less active therapeutically than metamidium, it was no less active prophylactically. The local reactions produced by the subcutaneous injection of 10 per cent w/v aqueous suspensions of *M & B* 4596 into calves compared favourably with those caused by metamidium chloride and metamidium suraminate.

The prophylactic activity of *M & B* 4596 against *T. congolense* has been confirmed in cattle, and results of field-trials in Africa are very encouraging. The compound has also been presented for examination as its 12 per cent w/v water-soluble methane sulphonate (*M & B* 4596*B*), and its sparingly water-soluble β-naphthalene sulphonate (*M & B* 4596*A*).

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ANATOMY

Staining Properties of Stressed Bone

The recent communication by Prof. C. J. Dreyer[1] on this topic is of great interest in relation to the general matter of the staining reactions of (presumably) collagen fibres. The change in staining reaction from green to reddish-brown with a corresponding change from optical isotropy to anisotropy, in unstressed and stressed bone, respectively, accord well with what has been observed in this Department in work on developing collagenous tissues. Using the Falg technique[2,3], it has been found that there are three stages in the development of this tissue which may be called the initial, the semi-mature and the mature. These three stages are distinguishable with the greatest constancy by the readiness with which they accept acid fuchsin, that is, by what has been earlier termed their erythrophilia. In the initial stage, presumptive collagen fibres are weakly erythrophile, in the semi-mature stage they are moderately erythrophile, and in the mature stage they are strongly erythrophile. The Falg technique, as well as others based on a similar chemical process, enables the consequent differences in monochromatic coloration (amplitude) to be converted into corresponding differences in colour (wave-length). Weakly erythrophile elements (of any kind) are coloured blue, the moderately erythrophile are coloured violet and the

strongly erythrophile are coloured a deep red. In these reactions the acid fuchsin, which is an amphoteric and not an acid dye, behaves as an acid towards the basic elements of the tissues stained by it. Other acid dyes, for example, light green yellowish *SF* in the Falg technique, attach themselves to the acid fuchsin basic side-chains in a manner discussed in the papers cited.

The Masson stain used by Dreyer is a very complex staining mixture, for it includes not only acid fuchsin and aniline blue (an acid triphenylmethane behaving like the other acid triphenylmethane, light green) but also hæmatoxylin. Recent work in this Department shows that hæmatoxylin will attach itself to free acid groups of the acid dye which has attached itself to acid fuchsin as mentioned above. Nevertheless, in all cases mature collagen fibres retain the red colour. Accompanying the increased erythrophilia of developing collagenous tissues there is a corresponding increasing birefringence, particularly well shown by the compound polarizer, the principles of which have already been stated in *Nature*[4].

It would appear then that Prof. Dreyer's results are explainable as a demonstration of a special maturation of collagen fibres in those regions of stressed bone which he has studied. This is in accord with the general principle that collagen fibres are to tension fields as iron filings are to magnetic fields[5], a principle well established both by gross and microscopic anatomy on one hand and experiment on the other.

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PATHOLOGY

Experimental Atheroma in Budgerigars

Since Anitschkow and Chalatow[1] in 1913 first induced lesions simulating human atherosclerosis in rabbits by feeding a diet rich in cholesterol, many workers have used rodents for this purpose despite the fact that spontaneous atheroma, apart from microscopic lesions, rarely develops in these mammals. Experimental atheroma has been produced by various dietetic alterations in other animals, including monkeys[2,3], pigs[4], chickens[5,6] and geese[7], but the parrot family (Psittacidae) have rarely been used although the more aged, captive birds in this order develop spontaneous atheromatous-like arterial lesions of an intensity usually seen only in man[8,9]. This communication describes cholesterol atheromatosis induced in budgerigars (*Melopsittacus undulatus*), which are the least expensive and most easily reared and housed of the psittacines.

Twenty-eight adult female budgerigars, reputably aged between 2 and 5 years, were divided into four equal groups. Group 1, used as controls, were fed a diet of 60 per cent millet and canary seed and 40 per cent pin-head oatmeal. Group 2 received 60 per cent seed, 38 per cent oatmeal and 2 per cent cholesterol. Group 3 received 60 per cent seed, 23 per cent oatmeal, 2 per cent cholesterol and 15 per cent Danish lard. Group 4 were fed a diet of 60 per cent seed, 25 per cent oatmeal and 15 per cent Danish lard. Food, water and grit were available *ad libitum*. One bird in each group was killed at 30-day intervals over a period of six months. Three birds died during the experimental period. Tissues were examined by naked-eye and histologically, and in order to facilitate recognition of small lipid deposits in the gross aorta and brachio-cephalic arteries the opened vessels were treated with sudan IV.

Fig. 1. Atheromatous plaque containing numerous foamy, lipid-laden histiocytes in aorta of a budgerigar fed on a diet containing cholesterol and lard for 4 months. Due to inversion the vessel lumen is at top left. Hæmatoxylin and eosin staining. (× 65)

Birds in groups 2 and 3 showed slight, sudanophilic lipid stippling of the inner walls of the aorta and brachiocephalic arteries after receiving their respective diets for two months. After three months, multiple, creamy white or yellow, lipid-containing nodules and plaques had formed in the intima and inner media of the arteries (Fig. 1). These lesions were more marked and more extensive at the end of the present experimental period of six months. Sub-endothelial accumulations of lipid narrowed several of the small coronary vessels (Fig. 2) and, in two instances, related small areas of myocardial infarction were seen. Vascular thrombosis was not observed. Blood cholesterol-levels were raised in all birds of groups 2 and 3; concentrations being greater than 300 mgm. per ml. as compared with cholesterol-levels

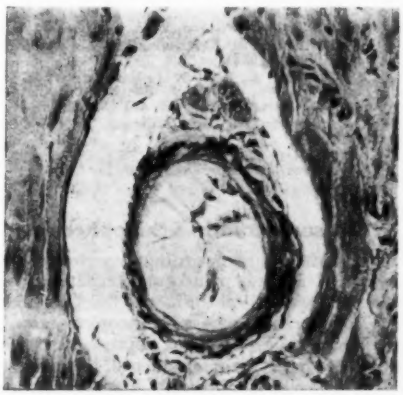

Fig. 2. Sub-endothelial lipid in small, intramural coronary vessel. Lumen distorted and narrowed. Weigert's elastic and van Gieson's stain. (× 300)

ranging from 150 to 200 mgm. per 100 ml. in group 1 (control) birds. The vascular changes were slightly more marked in group 3 as compared with group 2, but considering the small number of animals used this may well have been due to chance. No vascular lesions were observed in birds of group 1 and group 4, except for some light sudanophilic speckling of the thoracic aorta and brachio-cephalic trunks, associated histologically with small, focal lipid deposits in the non-muscular laminæ of the inner media of the arteries, of one bird in group 1.

The livers of birds in groups 2 and 3 were enlarged, pale and fatty. Initially the lipid infiltration was diffuse and largely within the cytoplasm of parenchymal hepatic cells; later the lipids accumulated more focally and appeared to be mainly within the cytoplasm of histiocytes. Lipid-laden histiocytes were seen also in the spleen, kidneys and ovaries. Birds in group 4 showed moderate fatty changes in the liver during the first three months of the experimental period. The liver and other viscera of birds in group 1 were normal.

Although experimentation has been limited, it would appear that diets containing added 2 per cent cholesterol or 2 per cent cholesterol and 15 per cent animal fat in the form of lard are highly atherogenic to young, adult female budgerigars, whereas a diet supplemented only by 15 per cent lard does not produce vascular lesions. Further work on naturally occurring and experimental atheroma in psittacines is being undertaken. While the pig is probably the most satisfactory animal for studies on atherosclerosis, it is suggested that the budgerigar may be of value where space and money are restricted.

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Orosomucoid in Schizophrenia

Orosomucoid, a glycoprotein extremely rich in carbohydrate, is the principal constituent of the perchloric acid-soluble seromucoid fraction of human serum[1]. In contrast to the seromucoid fraction which contains several proteins and represents more than one biological entity, orosomucoid is homogeneous and behaves as a single protein. With the production of specific chicken antiorosomucoid sera[2,3], it has become possible to investigate the clinical significance of orosomucoid. Interest in doing so arises from the fact that elevations of seromucoid and total serum glycoprotein-levels occur in a number of diseases; for example, in malignancy, tuberculosis, rheumatoid arthritis, and multiple sclerosis[4]. Elevations in this chemically defined group of proteins also occur in response to a wide range of non-specific stressors such as fractures, epinephrine injection, and burns[5].

Serum-levels of orosomucoid have now been measured immunochemically[2,3] in a large number of cancer patients and to a smaller extent in patients with other organic pathologies. Quite marked elevations of the protein have been observed, particularly in advanced malignancy[3], suggesting that processes destructive of tissue play a key part in increasing its serum concentration. However, such processes may merely serve as non-specific stressors which then act through systemic mechanisms to increase the serum concentration of orosomucoid. This communication concerns orosomucoid in schizophrenia, a disease in which tissue pathologies have never been demonstrated.

Comparisons were made between 41 non-psychotic control subjects (laboratory personnel) and 40 schizophrenic patients with respect to serum-levels of orosomucoid and albumin. These proteins were determined as in refs. 3 and 6, involving the precipitin reactions of specific chicken antisera. In previous work with cancer and tuberculosis sera, it was observed that when the orosomucoid concentration was markedly elevated, the albumin concentration was depressed. Thus the ratio of orosomucoid to albumin seemed to provide an even more sensitive indication of a pathological process than the orosomucoid-level alone. It was for this reason that both orosomucoid and albumin were measured in the controls and schizophrenics and that the orosomucoid : albumin ratio was computed for each subject.

Both the control and patient groups were off drugs and were free of physical disease so far as could be determined by physical examination, chest X-ray, routine complete blood count, and urinalysis. All the patients had been in hospital with schizophrenia for more than 4 years. Two-thirds of the group were under careful dietary supervision in a metabolic ward at the Lafayette Clinic, while the remaining third came from Pontiac State Hospital. The data for these two patient groups were combined, since there were no significant differences between them in the variables under investigation.

Table 1. Comparison of Albumin- and Orosomucoid-Levels in Schizophrenic and Control Groups

	Control group ($N = 41$)		Schizophrenic group ($N = 40$)			
	Mean	*S.D.*	Mean	*S.D.*	*t*	*P*
Albumin	48·3	3·4	45·7	4·5	2·90	< 0·01
Orosomucoid	0·842	0·185	1·005	0·196	3·81	< 0·001
Ratio of orosomucoid to albumin	0·0175	0·0041	0·0223	0·0055	4·34	< 0·001

The mean albumin (Table 1) was significantly lower for the schizophrenic group than for the control subjects ($P < 0·01$). In contrast, the mean orosomucoid was significantly higher for the schizophrenic subjects ($P < 0·001$). The difference between the two groups was also evident when the orosomucoid/albumin ratios were compared: 0·0223 in the schizophrenic group against 0·0175 in the control group.

These findings are of interest in two ways: (*a*) they suggest that orosomucoid may be a reactor to a variety of non-specific stressors not necessarily

associated with tissue destruction or proliferation, and (*b*) they indicate that other than purely psychological factors appear in the chronic schizophrenic illness and that either primary or secondary metabolic changes have occurred. In this connexion the hypothesis that schizophrenic patients are in a chronically stressed state would seem to be supported.

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RADIOBIOLOGY

Activity of Deoxyribonuclease as related to Root-Growth

It has been repeatedly observed in the past few years that deoxyribonuclease (DNase) activity is increased in normal growing animal tissues[1–5]. As postulated by Brody[2], it is possible that the increase in acid DNase activity counterbalances the greatly enhanced activity of the deoxyribonucleic acid (DNA) synthesizing enzymes and indeed determines the normal character of the growth. Cancerous tissues, on the contrary, have a lower DNase activity, at least when the activity is expressed per mgm. DNA (ref. 2), but not, apparently, when it is expressed per mgm. nitrogen[6].

In plant roots, the mitotically active meristem can be easily separated from the upper regions where the mitotic activity is very much lower. Pea seeds (*Pisum sativum*) were allowed to germinate on paper soaked with distilled water for 3 days at room temperature. Roots of approximately equal length were then selected and cut in two fragments: the tip, 1·5–3 mm. long, and a longer fragment immediately above the tip. The mitotic indices determined on sections of each fragment after Feulgen staining gave values of 35–45 a thousand in the tip and less than 1 in a thousand in the upper fragment.

Fifty to hundred fragments of each kind of the same length are pooled and homogenized in cold distilled water in a Potter tube. A sample of the homogenate is pipetted for total nitrogen determination by the Kjeldahl method. The rest is used for measurements of DNase activity by viscosimetry at 37° C. (ref. 7). The activity of the acid enzyme is measured in acetate buffer 0·2 *M* *p*H 4·8 in the presence of sodium citrate (final concentration 0·017 *M*) and highly polymerized DNA (final concentration 0·4 mgm./ml.) prepared from calf thymus[8]. For neutral DNase, phosphate buffer 0·2 *M* *p*H 7·6 with magnesium chloride (final concentration 0·04 *M*) is used. 0·02 ml. 'Triton-*X* 100' 10 per cent is added just before introduction of the mixture into the viscosimeter. The activity is expressed by the initial rate of decrease of the specific viscosity extrapolated to 10 min., or by the actual decrease in 10 min. when a straight-line relationship with time is obtained. For determination of the DNA content, at least one hundred root segments, cut exactly as for DNase assays, were homogenized in 70 per cent ethanol and processed according to the method in ref. 9; a small modification was introduced and consisted in incubating the delipidated tissue in cold 2 *N* perchloric acid for 18 hr. in order to get a better extraction of the ribonucleic acid (RNA). A diphenylamine reaction (Burton[10]) was performed on the hot perchloric extracts. A sample of the alcoholic homogenate was pipetted for nitrogen determination: the expression of DNase activity/mgm. DNA rendered it indeed necessary to make a correction for any small differences in nitrogen content which might occur between the segments selected for DNA determinations and those selected for enzymic assays.

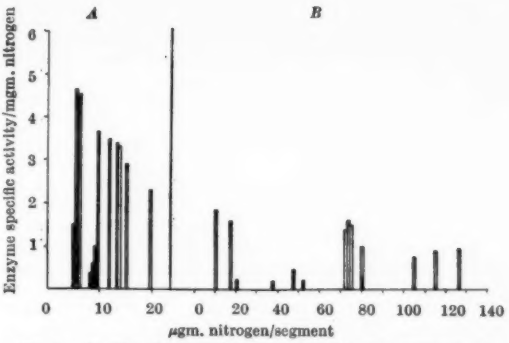

Fig. 1. Specific DNase activity in two different segments of the pea root. *A*, Tip of root; *B*, upper fragment (see text); white bars, acid DNase; black bars, neutral DNase

In Fig. 1, the specific activity of DNases/mgm. nitrogen is given for the tip of the root (part *A*) and for the upper fragment (part *B*). The length of the segment is indicated by the values of total nitrogen/segment, in μgm., on the abscissæ. It is clearly seen that the specific activity of acid DNase is much higher in the tip than in the other part of the root; the shorter the tip fragment, the higher the specific activity. Neutral DNase is also higher in the tip, but the difference between the two fragments is less marked than for the acid enzyme.

In Table 1, the specific activity of acid DNase expressed per mgm. nitrogen can be compared with the specific activity expressed per mgm. DNA in four experiments.

The ratio of enzyme activities between the tip and the upper part of the root ranges from 1 to 2·58 when the activity is expressed per mgm. DNA and from 2 to 6·65 when it is expressed per mgm. nitrogen.

Table 1. DNA and Nitrogen Content and DNase Activity in Two Different Fragments of the Pea Root

	μgm. DNA/segment	μgm. nitrogen/segment	Acid DNase/segment	Acid DNase/mgm. DNA	Acid DNase/mgm. nitrogen
Tip	1·9	13·8	0·042	22·5	3·05
Upper part	6·7	75	0·124	18·5	1·53
Tip	2·8	19·8	0·045	15·6	2·27
Upper part	6·2	115	0·098	15·6	0·84
Tip	1·72	13·4	0·046	26·8	3·41
Upper part	10·5	126	0·109	10·4	0·86
Tip	1·32	9·8	0·036	27·3	3·66
Upper part	4·60	103	0·058	12·6	0·55

Thus, as in many animal tissues, the acid DNase seems also linked to the process of growth in pea roots. Of course, many syntheses are going on at a high rate in the meristem, for example RNA, protein and DNA syntheses[11], but the metabolism with which the DNase is more probably connected is the DNA metabolism. It is known, however, from ref. 12 that DNA synthesis goes on in the older cells above the meristem and that there is up to four times more DNA per cell during elongation, without mitosis. This fact has been confirmed on *Vicia faba* roots[13]; it was observed by autoradiography that ^{3}H-thymidine was incorporated into the nuclei up to 7 mm. behind the meristem, with decreasing intensity.

Therefore, the decreased activity of acid DNase in the upper part may be a mere reflexion of a lower rate of DNA synthesis, or may indicate that DNase is also connected with the mitotic process and markedly decreases because the mitotic activity shows a dramatic drop above the meristematic part of the root.

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Enterohepatic Circulation of Bilirubin

The enterohepatic circulation of bile pigment has been the subject of protracted debate. Recirculation of bilirubin, and its bacterial reduction products, the urobilinoids, has been postulated and denied[1]. The availability of bilirubin labelled with carbon-14 in this Laboratory[2] has obviated many of the problems previously inherent in the study of bilirubin metabolism. The following is a preliminary report of investigations of the enterohepatic circulation of bilirubin.

Radioactive crystalline (unconjugated) bilirubin was prepared by methods described previously[2]. Radioactive conjugated bilirubin (in bile) was prepared by infusing crystalline labelled bilirubin intravenously into rats with an external bile fistula. Essentially all the injected label was excreted in bile as conjugated labelled bilirubin during the ensuing 3-hr. period.

For work on intestinal absorption, crystalline labelled bilirubin was dissolved in aqueous taurocholate, human albumin solution, or normal rat bile prior to administration, while conjugated labelled bilirubin was administered in bile. The labelled bile pigments were infused through a transoral duodenal feeding tube into each of 9 rats with an external bile fistula. The rats were allowed to recover from anæsthesia, and maintained, thereafter, in restraining cages permitting complete separation and recovery of excreta. After infusing the labelled pigments into the duodenum, collections of bile, urine, and faeces were made at regular intervals for determination of total radioactivity. Bilirubin was crystallized[2] from selected pooled samples of bile and the specific activity determined. At the termination of each experiment animals were autopsied to ascertain the absence of operative complications and the persistence of the bile duct catheter *in situ*.

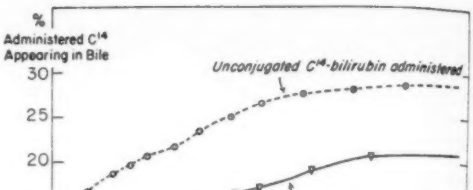

Fig. 1. Appearance of radioactivity in bile following intraduodenal infusion of either conjugated bilirubin (▽——▽) or unconjugated bilirubin (○ - - - ○) labelled with carbon-14

Table 1. Radioactivity recovered after Intraduodenal Administration of Conjugated and Unconjugated Bilirubin labelled with Carbon-14

	Infused radioactivity recovered in excreta (per cent)	
	Conjugated ^{14}C-bilirubin	Unconjugated ^{14}C-bilirubin
Bile	21	28
Urine	2	trace
Faeces	40	32
Total	63	60

Data based on radioactivity recovered in two rats during the 72-hr. period following intraduodenal administration of either 0·13 μc. conjugated labelled bilirubin or 0·25 μc. unconjugated bilirubin in a final volume of 2 ml. at similar bilirubin concentrations.

Representative results are given in Fig. 1 and Table 1. In all instances, significant intestinal absorption and biliary excretion of the carbon-14 label were evident. After the intraduodenal administration of both unconjugated labelled bilirubin and conjugated labelled bilirubin, much of the demonstrable absorbed radioactivity appeared in the bile as conjugated labelled bilirubin. The initial phase of absorption was more rapid for unconjugated bilirubin than for conjugated bilirubin and the total absorption of the former appeared somewhat greater. Little radioactivity was recovered in urine.

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BIOLOGY

Effect of Pupal Brain Surgery on the Rocking Response of *Automeris*

THE rhythmic rocking responses of Hemileucine moths may be released from insects which have been subjected to quite drastic surgical procedures[1], but they have never been observed in decapitated preparations[2]. An attempt has now been made to investigate the role of the brain in mediating this response and in determining its quantitative strength, using the techniques of pupal surgery described by Williams[3]. One hundred female pupæ of *Automeris cinerea* were chilled at 6° C. for eight weeks immediately following their reception from Argentina, and were then transferred daily (at 25° C.) in batches of five in order to break diapause. On the fifth to eighth day after transfer, they were chilled at 4° C. for 1 hr., anæsthetized in carbon dioxide for 30 min. while packed round with melting ice, and subjected to gross brain surgery through a window cut in the facial cuticle. After surgery, the pupæ were sealed with the usual precautions[2] and returned to 25° C. The yield in terms of histologically acceptable lesions was approximately 50 per cent. The surgical procedures, which were previously rehearsed on the large Indian *Antherea mylitta*, are summarized in Fig. 1. It may be noted that while procedure 1 results in blind moths the optic glomeruli II and III of which remain intact, procedure 2 ablates the entire optic complex, while sparing the medial lobes of the protocerebrum. Procedure 5 permits the development of a recognizable subœsophageal unit in isolation; while the resulting neuropil shows evidence of condensation, the normal features of its intrinsic organization may readily be recognized in favourably oriented sections, and moths so treated differ characteristically in their behaviour from those from which the subœsophageal ganglion has been extirpated.

Some 12 hr. before emergence was due, and just after the absorption of the moulting fluid, each surviving moth was dissected from the pupal case,

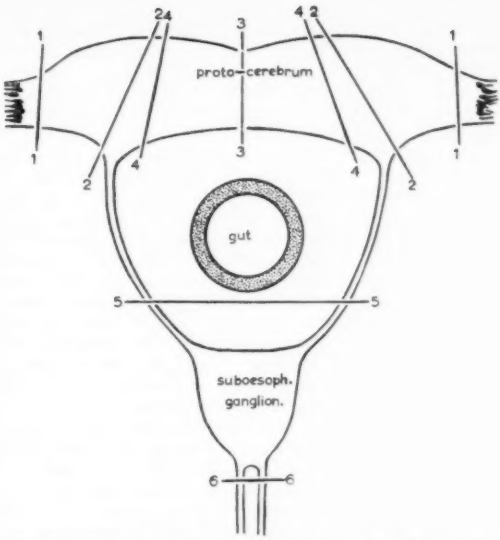

Fig. 1. Diagram showing the pupal brain, and the six surgical procedures

and its settling behaviour examined by the techniques which have been described in ref. 2; some individuals were kept at 20° C. and tested at intervals. The rocking response of *A. cinerea* does not show the quantitative stability of that of *A. aurantiaca*[2], and repeated testing at 5-min. intervals typically yields a range of scores lying between 10 and 45. The brains of all moths were dissected out, fixed in Dubosq–Brasil solution, sectioned at 10μ, and impregnated by a modification of the Holmes[4] technique, similar to that of Larsen[5] but using 2,6-lutidine in place of pyridine.

The results are summarized in Table 1; the left-hand column records the effective surgical procedure as scored at histological examination. The highest

Table 1

No.	Effective surgical procedures Left	Medial	Right	Score at dissection from pupa	6 ± 4 hr.	Adult scores 12 ± 4 hr.	24 hr.–5 days
1	1		1	(Spontaneous eclosion)	24:22:28*	—	—
2	1		1	26:33:38*	—	—	23:23
3	1		1	16:22:26:30*	—	—	22:23
4	1		1	12*	11:12:10	—	—
5	1		2	(Spontaneous eclosion)	24*:6	—	—
6	1		2	18:22*	—	—	—
7	2		2	41:42*	27:24:16:27:32	—	—
8	2		2	12:12:14:14:14	—	—	18:12:12:10:12:12:37*
9	2		2	33*:32	—	—	—
10	2		2	15	—	9:31:32*	—
11	2		2	(Spontaneous eclosion)	—	15:15:15:15:23:33*:18:6:15	—
12	2		2	33:34*	—	—	—
13	2		2	32*	—	—	—
14		3		30*	10:19:25	—	—
15		3		15:37*32	—	—	—
16	2	3	2	20:22:24:22:25:34:45*	16:38	9:8:9 (after oviposition)	—
17	2	3	2	10:10:14:10:26*	—	—	—
18	2	3	2	36*:12:10:12:10	—	—	—
19		4		10:10:10:12:13:5:5:5	—	—	—
		(large part of medial lobes left intact)					
20–31		4		Rocking absent	Rocking absent	Rocking absent	—
32–36		5		Rocking absent	Rocking absent	Rocking absent	—
		(Brain removed)					
37–41		5		Rocking absent	Rocking absent	Rocking absent	—
		(Brain left *in situ*)					
42–48		6		Rocking absent	Rocking absent	Rocking absent	—
		(Brain left *in situ*)					
49		Dummy		(Spontaneous eclosion)	18:24*	—	—
50		Dummy		10*	—	—	—
51		Dummy		42*	34:34	—	—

scores for each moth are marked with an asterisk; they may be compared with the highest response strengths scored for each of seven females at first settling after spontaneous hatching (38; 36; 43; 34; 17; 42; 45), and with the scores yielded by 66 males aged 8–24 hr. when settling for the first time following a disturbance[6] (mean = 23·39, $\sigma = 11{\cdot}53$). Despite the striking abnormalities of behaviour characteristic of each lesion, the co-ordination of the rocking response was unaffected except by procedures 2 and 3 in combination, where the amplitude of the response was increased: the only moth which gave a weak response after procedure 4 showed oscillations of shallow amplitude. While this latter procedure necessarily ablates the medial protocerebral neurosecretory cells, and the corpora cardiaca and allata, procedures 5 and 6 eliminate the rocking response whether or not the brain and its associated organs are left *in situ*. The role of the protocerebrum cannot, therefore, merely be that of providing endocrine priming for the lower neural structures. Furthermore, while eserine potentiates the rocking response of intact moths, it is unable to cause its re-appearance in decapitated animals[6].

These results show that for *A. cinerea* (i) protocerebral structures are necessary for the facilitation of the rocking response, which cannot be performed in their absence; (ii) within the limits of these experiments the quantitative strength of the response is independent of input from the optic complex, and (iii) it is probably independent of transverse connexions at the protocerebral level.

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Role of Cell Division in Gastrulation of the Viviparous Toothcarp *Xiphophorus helleri*

Recently it was stated that morphogenetic movements in gastrulation of the viviparous teleost *Fundulus heteroclitus* occur in a normal fashion in cases in which cytokinesis is prevented by treatment with the antimitotic alkaloid colchicine[1]. To establish whether a more general significance should possibly be attached to this phenomenon a corresponding investigation was performed with a fish bearing live young, namely the viviparous cyprinodont *Xiphophorus helleri*, the swordtail. For this purpose pregnant females in the first phase of gestation were injected intraperitoneally with 0·5 ml. of 0·001 *M* colchicine solution. During gastrulation in stage 12 (beginning gastrula), 13 (advanced gastrula), 14 (further advanced gastrula) and 15 (closure of blastopore) both treated and control females were killed by decapitation and the ovaries were fixed in Bouin's fluid. The blastoderms were sectioned at 5μ and stained with Heidenhain's iron hæmatoxylin. The mitotic index, representing the ratio of cells in mitosis to the total number of cells, and expressed as per cent, was determined in each blastoderm on the basis of cell counts of the sections.

Table 1. Mitotic Indices of Colchicine-treated and Control Embryos of *Xiphophorus helleri*.

Stage 12 = beginning gastrula, 13 = advanced gastrula, 14 = further advanced gastrula, 15 = closure of blastopore

Stage	Mitotic index (per cent) Colchicine treatment	Control
12	6·9	2·2
13	15·3	2·4
14	28·4	3·0
15	35·6	3·7

As in *Fundulus*[1], also in *Xiphophorus* the colchicine treatment did not have any apparent effect on gastrulation, which was similar to that in control blastoderms and consequently morphologically normal. The colchicine-treated blastoderms showed typical *c*-mitoses[2]. On the strength of mitotic index determinations for control and colchicine-treated blastoderms it could be established that in the treated blastoderms the number of *c*-mitoses increased, indicating that mitosis was completely blocked (Table 1). Thus, in stage 15, 35·6 per cent of the cells are in mitosis, while the mitotic indices of control blastoderms are relatively low during gastrulation. Consequently, also in early morphogenesis of viviparous teleosts cell division can be dismissed as a causative factor and apparently provides only material for morphogenetic movements[3]. This result, corresponding very well with the findings of Kessel[1] obtained with *Fundulus* and consequently of more general importance, is an argument for the view that gastrulation is a problem of mass movements and not of growth[4,5].

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Oxidase Activity in the Saliva of a Plant-Bug

In an account[1] of the origins of the salivary materials of a lygæid, *Oncopeltus fasciatus* (Dall.), it was reported that an oxidase other than cytochrome oxidase occurs in the accessory gland of the salivary apparatus, but not in the principal gland. No direct evidence was put forward for the discharge of the oxidase during salivation, but it was stated that the stylet-sheath material shows non-enzymic oxidizing properties that are not apparent in the precursors of the sheath material in the anterior and lateral lobes of the principal gland. It was therefore suggested that an oxidase from the accessory gland acts on the precursors as they are discharged. However, other evidence put forward suggested that the secretion of the accessory gland is a normal constituent of the watery saliva, which can be discharged independently of the sheath material. Evidence for the existence of an oxidase in the watery saliva of another lygæid, *Aphanus sordidus* Fabr. (the peanut trash bug or groundnut bug), has now been obtained, together

with further evidence for the oxidation of the sheath precursors as they are discharged.

The principal and accessory salivary glands of *Aphanus* were dissected into insect Ringer's solution and immediately transferred to Burstone's oxidase reagent[2], with or without inhibitors. In the absence of inhibitors, the cells of the entire salivary apparatus coloured within half a minute; in the presence of 0·003 *M* potassium cyanide, there was no reaction; and in the presence of 0·003 *M* sodium azide, the whole of the accessory gland and the principal salivary duct, but no other part of the salivary apparatus, were coloured. In view of Burstone's report[3] that cytochrome oxidase is inactivated if tissues are left in physiological saline for 30 min., it was interesting to observe that salivary glands which had been left in Ringer's solution overnight and were then transferred to the oxidase reagent showed the same reaction as fresh glands incubated in the reagent plus azide; that is, only the accessory gland and salivary duct were coloured. When the lobes of the principal gland were ruptured in the reagent, either with or without azide, the emerging contents did not become coloured and no coloured precipitate was formed.

Watery saliva was collected from the insects by forcibly extending the rostrum and collecting the liquid which exuded from the tip. The salivation of bugs in these circumstances was erratic: often no saliva could be collected, but sometimes a bug appeared to salivate so freely that the whole of the rostrum and head capsule became bathed in liquid. To provide against the possibility that the latter phenomenon was due to bleeding caused by damage to the bug during handling, tests for oxidase activity were made both on the liquid assumed to be watery saliva and on blood withdrawn from the insect by puncturing an abdominal intersegmental membrane with a fine pipette.

When the collected saliva was discharged into a drop of reagent on a white tile, a purple-blue precipitate formed, usually within a few seconds, and a blackish-purple border began to form around the drop of reagent within 5 min. In contrast, the reagent alone left only a pink residue when it evaporated. The insect's blood gave no reaction. When the saliva was discharged into the reagent diluted with an equal volume of 0·005 *M* potassium cyanide, a slow, weak reaction occurred, and there was no reaction if the saliva was first mixed with its own volume of cyanide and then discharged into the reagent. On the other hand, when the saliva was first mixed with 0·005 *M* sodium azide or with water, a rapid reaction occurred.

Sheath material was collected as the 'collar'[4] left at the surface of feeding punctures on peanuts. In the oxidase reagent, the sheath material developed no more than a faint pink colour, and there was thus no evidence for oxidase activity in the material itself. However, experiments with oxidation-reduction indicators showed that the sheath material had a higher oxidation-reduction potential than the contents of any of the lobes of the principal gland. A satisfactory indicator was 0·01 per cent thionin blue in veronal-acetate buffer *p*H 7·0. Before use, it was reduced by the dropwise addition of 1 per cent sodium dithionite until the indicator was almost colourless. A salivary gland was dissected into a drop of insect Ringer's solution made 0·005 *M* with respect to potassium cyanide, and pieces of fresh sheath material were placed alongside. All the lobes of the principal gland were punctured and the whole was enclosed under a coverslip in the reduced thionin. The sheath material and the walls of the gland immediately became coloured, whereas the contents of the gland, which were observed both as they emerged and after some had coagulated, remained colourless. On the other hand, when the indicator had first been re-oxidized by exposure to air, the contents of all the lobes stained to some extent, although the contents of only the anterior lobe approached the same intensity of staining as the sheath material.

These experiments showed that an oxidase other than cytochrome oxidase is present in the accessory salivary gland and in the salivary ducts of *Aphanus*; that a similar oxidase is present in the watery saliva; and that the stylet-sheath material has a higher oxidation-reduction potential than the contents of any lobe of the principal gland. The results are consistent with the view that an oxidase acts on the sheath precursors as they are emitted; however, the presence of a similar enzyme in both the accessory gland and the salivary duct is a complicating feature, since either or both parts of the glandular system could provide the oxidase in the watery saliva and an enzyme which oxidizes the sheath precursors. Possibly both parts are functionally similar, and the accessory gland should be considered as purely a development of the salivary duct, as suggested by Baptist[5].

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Pre-Harvest Prophylaxis for Infestation Control in Stored Food-Grains

Stored food-grains suffer from considerable losses due to insect infestations in the tropical and sub-tropical regions of the world[1]. During post-harvest storage, cross-infestation by insect pests and the multiplication of pests as a result of field-infestation occur in stored grains even within a storage period of a few months in warehouses[2–3]. Fumigation with noxious gases such as methyl bromide, carbon disulphide, carbon tetrachloride, hydrocyanic acid, ethylene oxide and others, although of use in the control of most infestations, is not easy for the producer or the warehouse-man to apply[2]. Parkin[1] observed that application of such measures unfortunately lags behind the advance of knowledge in all but the most highly developed countries, and even in those is not always so widespread or up-to-date as it could be. A new approach has therefore been made to replace the fumigation treatment with a relatively simpler prophylactic spray treatment of the grain under pre-harvest condition, using a chemical of low toxicity for mammals, for the control of pest infestation during post-harvest storage.

Canada and the United States have set a tolerance limit of 8 p.p.m. for malathion in stored food products[4]. The U.K. Ministry of Agriculture, Fisheries and Food has permitted the admixture of malathion with raw unprocessed grains for their storage. Godavari Bai *et al.*[4] observed that more than 50 per cent of malathion from wheat flour was lost in one month through *A*-twill bags. The average concentra-

tion of malathion was less than 2 p.p.m. after two months storage. At this concentration, malathion could exert little prophylactic action on pests of stored grain. The possibilities of disinfesting grain under field conditions with malathion spray at its permissible dosage and affording longer protection from cross-infestation by storing it in sacks impregnated with insecticides, were therefore investigated.

The panicles of sorghum crop (*Sorghum vulgare*) were sprayed with malathion (Premium grade, American Cyanamid Co.) water-dispersible emulsion at various dosages on different plots. The grain was harvested, threshed and stored in lindane-impregnated *B*-twill sacklets. Grain samples were analysed for malathion by the method of Norris *et al.*[6]. Sorghum harvested from the control plot was stored in impregnated and unimpregnated sacklets and, for comparison, control grain was fumigated and stored in these bags. Experimental grain sacklets were incubated in an infestation room having large populations of *Sitophilus oryza* (Linn.) and *Tribolium castaneum* (Hbst.) for 150 days.

The results of the experiment, shown in Table 1, demonstrated that this simple treatment can replace the fumigation treatments for pest control in stored grain.

Table 1. Effect of Pre-harvest Spraying of Malathion on the Post-harvest Storage of *Sorghum vulgare*

Treatment	Malathion concentration in p.p.m. after 3 days of spraying	Living adults (No./kgm.) after 150 days incubation at 24–28° C. and rel. hum. 48–83 per cent			
		T. castaneum		*S. oryza*	
		Impregnated*	Control	Impregnated*	Control
Pre-harvest malathion spray	1·9	2	8	0	63
	2·4	1	3	0	21
	7·2	0	2	0	8
	7·7	0	1	0	12
	14·2	0	0	0	0
Control	0·0	3	29	49†	283
Fumigated‡	0·0	0	32	0	308
Post-harvest malathion spray	8·1	0	2	0	9

* Impregnated bag at 75 mgm. lindane/sq. ft.
† As a result of field-infestation.
‡ Grain fumigated with 32 mgm./litre methyl bromide for 48 hr.

In most cases stored food-grains are fumigated in warehouses only when they show open signs of insect infestation, for untrained warehouse-men, in the tropics and in many other parts of the world, cannot detect incipient infestation. On the other hand, field-spraying of crops with a pesticide is much more commonly adopted throughout the world. The newer pesticidal substances having differential toxicities[7] to insect pests and mammals, such as malathion, present a unique means of introducing simple pest control treatments for stored food-grains. Spraying of malathion on the crop before harvest at a required level to obtain a concentration below the permissible limit in the threshed grain and storing in an insect-proofed structure or jute sacks can afford satisfactory protection from pest infestation in post-harvest storage. Treatment of harvested grain with malathion would require elaborate machinery for spraying the regulated grain-flow on a conveyor, but applications in the field on the crop would be easy. This new approach to pest control problems of stored food-grains probably offers a simpler solution for large-scale application where fumigation with toxic gases cannot be easily applied. Although malathion is rapidly dissipated from the treated grains, the possible residual toxicity to consumers, of traces of the chemical on food-grains, needs further assessment in the present context.

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Perennial Nodules on Native Legumes in the British Isles

It is generally maintained that the normal life span of legume nodules in temperate climates is restricted to one season or growth cycle of the host plant. Frost, drought[1] and insect damage[2], or developmental changes in the host such as flowering and fruiting[3], are recognized agencies leading to destruction of nodules.

Nodules on certain tropical woody legumes and perennial species of *Lupinus* are classed as exceptional in developing annual or seasonal increments of bacterial tissue. Various anatomical features are associated with the prolonged activity of such perennial nodules[4].

In an earlier paper[5] a successful over-wintering of nodules was described as a common event in winter annual, biennial and perennial legumes in Northern Ireland. During four seasons of investigation (1952–56) involving 29 native species, a life-span of 10–20 months was recorded for nodules surviving into a second season. No evidence was obtained of nodules persisting into a third season of plant growth.

This communication reports the finding, in February 1961, of what are considered to be truly perennial nodules on the roots of gorse (*Ulex europaeus* L.) and broom (*Sarothamnus scoparius* Koch.) growing on drained peat bog near Randalstown, Co. Antrim. The nodules are multibranched structures measuring some 1–1½ in. in length and weighing as much as 1 gm. They are borne on thin fibrous roots traversing the upper layers of partially decomposed peat. Many show apogeotropic growth forms similar to those to be observed in root nodules of *Alnus* and various cycads.

The following findings support the view that the nodules are several years old and clearly equipped for at least another season of growth and nitrogen fixation:

(1) 3–6 annual growth rings can be counted in the parent roots of the larger nodules. This suggests that the nodules are of a similar age, since nodule formation in both gorse and broom occurs at infection sites underlying the piliferous layers of very young roots.

Fig. 1. Perennial nodules on native legumes in Britain. Upper row from broom, lower row from gorse. Material obtained from a drained peat-bog, near Randalstown, Co. Antrim, N. Ireland (×½)

(2) A mature nodule may show many constrictions along its length (Fig. 1). These constrictions apparently demarcate seasons of growth activity.

(3) Sectioning reveals several features reminiscent of those described for perennial nodules by other writers[4-6]. Outer layers of the nodule cortex develop heavy suberization in all mature parts of the nodule. Vascular tissue persists in healthy condition in older portions of the nodule, although all other inner tissues decay and may even disappear with age. Healthy meristems terminate each nodule lobe. Behind these occurs a zone of bacterial tissue pigmented with hæmoglobin. Adjacent older tissues show an accumulation of green bile-type pigments. The nodule cortex in these younger regions contains abundant starch, particularly in lines of cells adjacent to the vascular tissue. Paper chromatography of the soluble-nitrogen fraction reveals that aspartate, glutamate, and their respective amides, asparagine and glutamine, accumulate in quantity in the nodule. The latter amides and starch are the principal storage products in other parts of the over-wintering plants.

It would be interesting to know whether perennial nodules are developed on native or introduced legumes in other parts of the British Isles. Nodules on gorse and broom are clearly equipped anatomically for perennation, yet this event may be achieved in Nature only after a series of mild winters, and possibly only in soil conditions where decay of older parts of the nodule is arrested.

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Cultivation of Goitrogenous and Non-Goitrogenous Cabbage

Results of earlier work on the goitrogenous properties of plants[1-3] supported the assumption that goitrogenous activity of vegetable foodstuffs, particularly that of *Brassica* species, depends to a large extent on the content of sulphur compounds as goitrogenous substances. Therefore, in an attempt to supply experimental evidence for the relationship between cabbage goitrogenicity and the intake and rate of utilization of sulphates from nutrient environment, cabbage (*Brassica oleracea*, var. *capitata*, subsp. Amager) was cultivated in pure silica sand (SiO_2), in vegetation pots, by means of nutrient solutions in which only the sulphate content was made to vary. The results of the first experiment in this series had confirmed the above assumption, namely, that goitrogenous activity of the cabbage increased in relation to the sulphate content in the nutrient solution[4,5]. However, efforts to grow cabbage without goitrogenous properties had failed. In the second experiment I endeavoured to grow both the goitrogenous and the non-goitrogenous variants of cabbage side by side. The same species of cabbage was again grown in vegetation pots with silica sand by means of nutrient solutions. The composition of the solutions for variants I and II is given in Table 1.

Table 1. Composition of Nutrient Solutions

Type of nutrient salt ions	Concentration of nutrient expressed in mgm. equivalents/l. of nutrient solution: Cabbage var. I	Cabbage var. II
Sulphate	1	25
Nitrate	25	23
Magnesium	8	8
Calcium	15	15
Phosphate	8	8
Potassium	12·66	12·66
Ammonium	0	2
Chloride	8	0
Sodium	1	10

Iron and micro-elements added in equal amounts expressed in mgm./l. of nutrient solution for both the cabbage variants

Iron	5·0 mgm.	(0·025 gm. of ferric citrate)
Manganese	1·0 mgm.	(0·00406 gm. $MnSO_4.4H_2O$)
Copper	0·1 mgm.	(0·0003928 gm. $CuSO_4.5H_2O$)
Zinc	0·1 mgm.	(0·0004397 gm. $ZnSO_4.7H_2O$)
Boron	0·5 mgm.	(0·002858 gm. H_3BO_3)
Molybdenum	0·02 mgm.	(0·000037 gm. $(NH_4)_6Mo_7O_{24}.4H_2O$)

Beginning with the second half of the growth period, all sulphates were withdrawn from the nutrient solution for variant I by leaving out 1 mgm.-equivalent of sulphate ion and sodium ion. The remaining nutrients, such as iron and microelements, continued to be supplied in equal amounts to the nutrient solutions for both the cabbage variants, as seen in Table 1. At the end of the period of growth the content of sulphur compounds was determined in both the variants. Total and inorganic sulphur, expressed in terms of sulphates, was determined gravimetrically, thiocyanates according to Aldridge's method[6], and SH-compounds polarographically according to Zuman[7]. The results are summarized in Table 2.

It was found that the content of total, inorganic and organic sulphur is several times greater ($P \leq 0{\cdot}0005$) in cabbage variant II than in variant I. Thiocyanate content of variant II was likewise significantly higher ($P \leq 0{\cdot}02$). The values for SH-compounds were significantly higher ($P \leq 0{\cdot}001$) in the green leaves of variant II than in those of variant I, while no differences were noted in the cabbage heads. In general, the content of sulphur compounds, with the exception of thiocyanate and SH-compounds, was markedly higher in the green leaves than in the cabbage heads.

The goitrogenous activity of the cabbage variants was assayed on rats, where the relative thyroid weights, the thyroidal iodine-131 uptake in 24 hr. and the level of protein-bound iodine-131 in serum were followed and a histological evaluation of thyroid preparations was made. A strong goitrogenous activity was observed in rats fed with cabbage variant II, while variant I failed to show any activity when the

Table 2. Results of Sulphur Compound Determinations in Cabbage

Type of test (n = 10)*	Variants of cabbage			
	I		II	
	Green leaves	Cabbage heads	Green leaves	Cabbage heads
Desiccated cabbage (per cent)	11·64 ± 1·48	8·36 ± 0·58	10·58 ± 1·76	8·46 ± 1·34
Total sulphur in mgm. per cent sulphate	46·6† ± 9·6	66·1 ± 29·5	427·3 ± 208·1	164·0 ± 45·6
Inorganic sulphur in mgm. per cent sulphate	17·3 ± 8·2	34·1 ± 29·1	322·8 ± 156·2	89·6 ± 42·5
Organic sulphur in mgm. per cent sulphate	29·3 ± 10·0	31·9 ± 12·9	104·5 ± 66·6	74·4 ± 26·7
Thiocyanate, according to Aldridge, in mgm. per cent SCN'	1·6 ± 0·51	2·14 ± 0·46	2·62 ± 1·12	2·68 ± 0·81
SH-compounds expressed in mgm. per cent SH-glutathion	3·82 ± 2·12	8·01 ± 2·74	9·12 ± 3·04	9·89 ± 1·36

* n, No. of determinations in the experiment.
† Average ± *S.E.*

results were compared with those from a control group of rats, which had received Larsen's diet only. The nutritional value of both the cabbage variants was the same, for I failed to notice any significant differences in the sugar content, vitamin C, total nitrogen and calcium values between the two cabbage variants.

The plant material as defined here, considered from the point of view of cultivation, and the possibility of cultivating goitrogenous and non-goitrogenous variants of cabbage side by side, offer new opportunities for experimental research in this field.

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Biologically Active Materials in Coal

That materials extractable from coal with polar organic solvents inhibit the growth of certain bacteria and fungi has been known for some years[1–5]. The name 'vitricin' has been applied to the group of biologically active substances found in coal[1]. Mills recently reported work on acetone and methanol extracts of coals of various ranks[5]. He found that bacteriologically active extracts contained acidic, basic, neutral, and phenolic components; attempts to separate active components by extraction of ethereal extracts with aqueous acids, alkalis or buffers met with little success. Testing the crude extracts against *Bacillus subtilis* in nutrient broth demonstrated bacteriostatic activity of the solute at a concentration of 30 μgm./ml. Mills did, however, by steam distillation of an acetone extract of Tupton coal (high-volatile bituminous), obtain an oil fraction which inhibited Gram-positive bacteria and certain moulds.

Research workers at the Federal Bureau of Mines prepared and investigated several methanol and acetone extracts of various coals. In agreement with previous workers[3–5], the most active extracts were obtained from high-volatile sub-bituminous or bituminous coals. Peat and lignites yielded lesser amounts, and anthracite practically no acetone- or methanol-extractable material. Contrary to Mills's observations, the most active material obtained was the 5 per cent aqueous sodium hydroxide-soluble fraction from ether solutions of materials obtained by acetone extraction of coals high in volatile matter. This material, recovered from the hydroxide solutions by precipitation with acid and centrifugation, was active in nutrient broth dilution tests against *Bacillus subtilis* at concentrations of 5–10 μgm./ml.

We recently obtained a large sample of material for more detailed study by treating 200 lb. of a high-volatile sub-bituminous *B* coal (Rock Springs, Wyoming) with 150 gal. of acetone. The work was carried out at room temperature on coal ground to pass a 200-mesh screen. When the solvent was evaporated, a brownish-black, soft tarry material remained. This represented about 1 per cent of the original weight of coal. Previous observations were confirmed with this extract.

A 400-gm. sample of the extract was subjected to steam distillation and 14 ml. (11·6 gm.) of a volatile yellow oil was obtained. The oil was separated into its acidic, phenolic, basic, and neutral components and these were tested in nutrient broth against *Bacillus subtilis*. Only minor activity was observed in the case of the acidic (partial inhibition at 100 μgm./ml.) and phenolic (partial inhibition at 40 μgm./ml.) fractions.

A 50-gm. sample of the residue from the steam distillation was cooled to − 68° C., crushed, and agitated for 5 min. in a Waring blender with 10 per cent (w/v) aqueous sodium hydroxide. The resulting suspension was mixed with a small amount of 'Celite' and then filtered. The filtrate was acidified with 6 *N* hydrochloric acid and extracted with ether continuously for 16 hr. This procedure yielded 6·4 per cent of the original residue. The hydroxide-soluble material inhibited *B. subtilis* at a concentration of 8 μgm./ml. in nutrient broth.

To confirm that phenolic materials were responsible for the observed activity at 8 μgm./ml., the trimethylsilyl ethers ($(CH_3)_3SiOR$) of the phenolic hydroxyls were prepared. A small sample of the hydroxide-soluble material was refluxed with hexamethyldisilazane for 8 hr. After refluxing, residual hexamethyldisilazane was removed by warming in a vacuum. The reacted material was active against *Bacillus subtilis* at a concentration of 40 μgm./ml. The reacted material then was refluxed for 6 hr. in ethyl alcohol (70 per cent v/v) to hydrolyse the trimethylsilyl ethers, and then heated (54° C.) *in vacuo* to remove the alcohol, water, and hexamethyldisiloxane formed during the reaction. The reconstituted material was active against *B. subtilis* at 8 μgm./ml. of nutrient broth and confirmed complete recovery of the originally observed activity.

The original acetone extract and samples obtained at the various concentration stages were analysed by mass spectrometry and were also examined in the ultra-violet. Mass spectrometer analysis of the total acetone extract showed peaks at mass units (in order of decreasing intensity) at 342, 324, 374, 376, 340, 350, and 392. These masses indicate a range of compounds in the six-ring series. Since ultra-violet spectra indicated a high percentage of three-ring systems, the

six-ring compounds detected by mass spectrometry probably consist of diphenanthryl, or similar systems. Control runs with acetone alone (no coal) demonstrated that polymerized solvent was not responsible for the mass peaks observed.

The acetone extracts differed mainly from the parent coal in elemental composition in having a higher hydrogen content. Analyses showed (per cent): Coal: C, 79·31; H, 5·41; N, 1·74; S, 0·90, O, 12·65; ash, 1·96.
Extract: C, 78·87; H, 9·92; N, 0·23; S, 0·21; O, 10·92; ash, 0·02.

Acetone might be expected to extract more polar compounds from the coal matrix, enriching the oxygen content of the extract, but apparently this did not occur. The oxygen content of the extract was 10·92 per cent, that of the whole coal 12·65 per cent. Perhaps materials soluble in acetone serve as solvents for other materials in the coal which are not acetone-soluble. On long contact with the coal, the extract then serves as a continuously changing solvent, contrasted to a soxhlet-type extraction, with resultant extraction from the coal of materials of high mass and low oxygen content.

Calculation of the amounts of hydroxide-soluble materials present in the original coal indicate a concentration of 150 μgm./gm. of coal. The material inhibits the test culture at 8 μgm./ml. of broth. It is possible that such materials play a part in the resistance of coal to biological oxidation, and the limitation of the coal flora[6] to non-susceptible forms.

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MICROBIOLOGY

Relationship between Phage Type and Host Plant in *Pseudomonas mors-prunorum* Wormald

Pseudomonas mors-prunorum causes bacterial canker of cultivated *Prunus* spp. in England and is widely distributed on the plum (*P. domestica*) and on the cherry (*P. avium*). Strains from plum and cherry show some degree of pathogenic adaptation to their original host[1], but have not been distinguished by routine physiological or biochemical tests. We now find that we can differentiate between them by phage typing.

The bacterial strains used in these experiments were collected over a period of 7 years from plum and cherry plantations within a restricted geographical area of about a half-mile square at East Malling Research Station in Kent. Bacteria were isolated from leaf surfaces, rain-water draining down the trees, and lesions on branches or foliage. The typing phages, with the original propagating host species in parenthesis, were as follows: *A*1, *A*2, *A*3, *A*7, *A*32 (*P. mors-prunorum*) and *S*3 and *S*8 (*P. syringae*). These phages were selected from a group of 57 isolated from soils by a method previously described[2]. The phages were purified by repeated selection from single plaques, and stored as high-titre preparations of approximately 10^{10}–10^{11} particles/ml. over chloroform at 4° C. In these conditions most of the preparations have remained stable for two years.

For phage-typing, 10^7 viable cells of the bacterial isolate under test were poured into plates with 12 ml. of a medium having the following composition: 0·3 per cent Difco yeast extract, 0·5 per cent Oxoid bacteriological peptone, 2·0 per cent glycerol, 1·0 per cent B.D.H. Japanese agar. When set, the plates were dried with the lids removed for 2 hr. at 30° C. and then spotted with 0·02 ml. of the phage preparation at the routine test dilution, that is, the lowest dilution giving confluent lysis on the original propagating strain. Finally, the plates were dried for about another hour and incubated overnight at 25° C. The results of tests on plum and cherry strains are summarized in Table 1 and compared with similar tests on *Pseudomonas syringae* isolates obtained from diseased pear blossoms in widely separated localities of south-east England during 1959–60.

Table 1. PHAGE-TYPING OF PLUM AND CHERRY STRAINS OF *Pseudomonas mors-prunorum* AND *Pseudomonas syringae* FROM PEAR

No. of bacterial isolates of each phage-type		Typing phages *A*1	*A*2	*A*3	*A*7	*A*32	*S*3	*S*8
(*A*.) *P. mors-prunorum* (1) from cherry:								
	14	+	+	+	+	+	−	−
	15	+	+	+	+	(+)	−	−
	2	(+)	+	(+)	+	+	−	−
Total 35	1	+	(+)	+	+	+	−	−
	1	+	(+)	+	(+)	(+)	−	−
	1	+	(+)	(+)	+	(+)	−	−
	1	+	+	+	+	(+)	−	+
(2) from plum:								
	6	+	−	*s*	−	−	−	−
	1	+	−	*s*	−	−	−	+
	1	(+)	−	*s*	−	−	−	−
Total 12	1	+	−	*s*	−	−	+	−
	1	+	−	*s*	−	+	−	−
	1	+	−	*s*	−	−	+	+
	1	(+)	−	−	−	−	−	−
(*B*) *P. syringae*								
	9	−	−	*s*	−	−	+	+
	1	−	−	*s*	−	−	+	(+)
	1	−	−	*s*	−	−	(+)	(+)
Total 22	2	−	−	−	−	−	+	+
	2	−	−	−	−	−	−	−
	2	+	−	*s*	−	−	+	+
	3	(+)	−	*s*	−	−	+	+
		s	−	*s*	−	−	+	+

+, Confluent lysis; (+), innumerable plaques; *s*, isolated plaques; −, no reaction.

Within each species or strain group there were different phage types. The plum strains of *P. mors-prunorum* differed from the cherry strains in failing to react with phages *A*2 and *A*7, and in giving no reaction or isolated plaques with *A*3. They were also less sensitive to *A*32. They resembled *P. syringae* in typing pattern, except that, as a group, they were more sensitive to *A*1 and less sensitive to the *P. syringae* phages *S*3 and *S*8. One plum isolate gave a typing pattern identical with one of the *P. syringae* isolates.

Phage sensitivity does not appear to be correlated with biochemical activities. All the *P. mors-prunorum* isolates tested, for example, were physiologically homogeneous and quite distinct from *P. syringae* in respect of the characters shown in Table 2.

Over the period of 7 years during which the *P. mors-prunorum* strains have been collected there have been no signs of any major change in the phage types associated with cherry or plum in the field. Some strains have been in culture for several years

Table 2. Biochemical Differentiation of *P. mors-prunorum* and Pear Isolates of *P. syringae*

Species	Æsculin and arbutin hydrolysis	Gelatin liquefaction	Casein hydrolysis	Tyrosinase activity	Growth in nutrient broth + 5 per cent sucrose
P.-mors-prunorum					
(*a*) Cherry strains	−	−	−	+	white
(*b*) Plum strains	−	−	−	+	white
P. syringae	+ *	+	+	−	yellow

* One isolate failed to hydrolyse æsculin and arbutin.

without passage through the host and appear, therefore, to be relatively stable *in vitro*. The difference between the plum and cherry strains may merely reflect a different history of phage-infection in the field. The cherry strains, for example, may have failed to acquire resistance to *A*2 and *A*7 or related phages because these are absent on cherry or their activity is in some way inhibited. Alternatively, the phage types may be a result of selection by the host plants. This would imply some form of association between phage sensitivity and specific virulence factors in *P. mors-prunorum*.

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Serotonin in the Small Intestine of Conventional and Germ-free Chicks

The function of serotonin (5-hydroxytryptamine) in the tissues of the small intestine is not understood even though it is found in relatively large quantities, particularly in the enterochromaffin cells[1]. The most frequently repeated interpretation ascribes control of intestinal motility and vascular permeability to serotonin[2,3]. Thus, if the tonus of the gut is dependent on serotonin-levels, germ-free animals, which possess poor tonus of the tract and distended cæca[4], might be expected to have a lower serotonin content in the gut than conventional animals. On the contrary, however, this communication provides evidence that intestinal serotonin-levels are actually depressed by the presence of micro-organisms in the lumen of the gut.

White Leghorn chicks were raised in a germ-free environment by methods similar to those previously described[5] on a complete starch-casein diet[6]. Fertile eggs were obtained from local hatcheries after 19 days of incubation, immersed in 2 per cent mercuric chloride solution for 10 min. at 39° C. and put into sterile 'Plexiglas' isolators where they hatched. Control chicks were treated in the same manner but were reared in a non-sterile isolator. After four weeks the chicks were decapitated; the small intestine was excised and washed in cold water. All germ-free animals were examined[7] for bacterial contamination when killed. The various segments of the intestine were homogenized in 0·1 *N* hydrochloric acid in a Ten Broek apparatus at 5° C., and serotonin was assayed[8] on fresh specimens in a Turner photofluorometer (G. K. Turner and Associates, Palo Alto, California).

Table 1. Serotonin in the Small Intestine of Germ-free and Conventional (Contaminated) White Leghorn Chicks at Four Weeks of Age

Treatment	No. of chicks	Serotonin (μgm./gm. fresh tissue) Duodenum	Ileum
Germ-free	20	9·0 ± 1·0 *	11·0 ± 0·7
Conventional	20	8·3 ± 1·3	6·1 ± 0·8
Significance of differences by the '*t*' test			
		t	*P*	*t*	*P*
Germ-free vs. conventional		0·84	< 0·4	8·8	< 0·01

* Mean ± standard deviation.

A significantly higher concentration of serotonin was found in the ileum of germ-free chicks than in control birds (Table 1). This is consistent with the observation[9] that rats and mice given peroral antibiotics produce significant increases of serotonin in the small intestine. However, no such difference was apparent in the duodenum (Table 1). There is insufficient evidence to determine whether these results reflect differences between the microflora of the duodenum and ileum or an influence of the intense microbial activity of the cæca[10,11] due to the proximity of the ileum to the latter. In any event, the presence of intestinal micro-organisms clearly affects serotonin in the ileum of chicks.

Several reports suggest alternative explanations for the microbial inhibition of serotonin. A few isolates of *Aerobacter aerogenes* and *Alcaligenes faecalis* appear to be capable of oxidizing serotonin *in vitro*[12] although it is not known if this event occurs in the lumen of the intestine. In addition, many species of lactobacilli which are present in large numbers in the chick[13] require pyridoxine or its derivatives[14]; pyridoxal phosphate is required as a co-factor in the decarboxylation of 5-hydroxytryptophan to serotonin[15]. Thus bacterial removal of the coenzyme may inhibit serotonin synthesis. A similar effect might be achieved by the interaction of a bacterial product with the coenzyme such as has been reported for norepinephrine and 3,4-dihydroxyphenylalanine[16].

Further work is required to determine the reason for the difference in intestinal serotonin between germ-free and conventional chicks and the physiological significance of this observation.

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VIROLOGY

A New Mutant of Dermovaccinia Virus

DURING the course of serial one-day egg passages of a strain of the chorioallantoic membrane-adapted dermovaccinia virus (*DIE* strain)[1], a new minute pock mutant was observed. This arose at the 13th one-day egg passage, when tiny pocks mixed with larger pocks typical of dermovaccinia were seen. The former increased in proportion during further one-day egg passages, and finally excluded the original virus. The mutant strain (*DIs*) was obtained in a pure form by two cycles of clone purification. These were initiated by a single pock produced on one chorioallantoic membrane by the inoculation of limiting dilutions of the fifteenth one-day egg passage. Some biological characteristics of this mutant were investigated and are described in this communication.

(1) *Serological specificity:* The strain *DIs* was neutralized specifically by antivaccinia rabbit serum, but no neutralization was observed by normal rabbit serum or by antiherpes simplex rabbit serum. When inoculated on to the chorioallantoic membrane of 12-day eggs, it produced vaccinia–common complement-fixing antigen and hæmagglutinin. These were separable from infectious particles by differential centrifugation. As the size of pock of the mutant resembles that produced by a strain of pigeon pox, comparative chessboard complement-fixation tests were carried out. The results summarized in Table 1 indicated no cross-reaction between the strain *DIs* and anti-pigeon pox–pigeon serum, though a slight cross was observed between the pigeon pox chorioallantoic membrane antigen and antivaccinia rabbit serum. This could be eliminated by ether treatment of the antigen.

(2) *Host range:* The strain *DIs* grows well in one-day eggs, on the chorioallantoic membrane of 12-day eggs, in the yolk sac of 7-day eggs and in the amniotic sac of 11-day eggs. No significant growth was observed, however, when it was inoculated into the allantoic cavity of 11-day eggs. When a large amount of the virus was inoculated intradermally into adult albino rabbits, it produced a slight reddening and flat swelling on the first few days, which then waned. The reaction was nearly the same as that observed with variola virus inoculated via the same route. No lesion could be seen following inoculation on to the scarified skin of rabbits. The mutant produced no swelling when inoculated into the rabbit testicle, no keratitis when inoculated on to the scarified cornea of rabbits. New-born, weanling and adult mice showed no clinical signs, when inoculated intracerebrally, intraperitoneally or subcutaneously. Infection of the scarified plantar skin of weanling and adult mice from an ectromelia-free colony failed to give rise to any local symptoms. No clinical symptoms were observed in newly hatched chicks inoculated via the intracerebral, intraperitoneal or subcutaneous routes. Roosters and guinea pigs produced specific antibody without any sign of clinical symptoms after several weekly injections administered via the subcutaneous or the intravenous route.

(3) *Physical properties:* The mutant showed the same resistance to ether as did the parent strain. The infectivity of the mutant was as stable as that of the original strain at low temperatures (−70° C. and 4° C.). Electron microscopy of the elementary bodies of the strain *DIs*, partially purified by fluorocarbon treatment, indicated that the shape and size of the elementary bodies were approximately the same as

Table 1. COMPLEMENT FIXATION TEST OF THE STRAIN *DIs* AND THE STRAIN NAKANO (PIGEON POX) AGAINST ANTI-PIGEON POX PIGEON SERUM

Antigen		Anti-pigeon pox pigeon serum							No serum
Code	Dilution	4	8	16	32	64	128	256	—
Nakano	6·3	4	4	4	4	4	tr.	0	0
	12·5	4	4	4	4	tr.	0	0	0
	25	4	4	4	tr.	0	0	0	0
	50	4	4	tr.	0	0	0	0	0
	100	1	tr.	0	0	0	0	0	0
	200	0	0	0	0	0	0	0	0
DIs	6·3	0	0	0	0	0	0	0	0
	12·5	0	0	0	0	0	0	0	0
	25	0	0	0	0	0	0	0	0
No antigen		0	0	0	0	0			

Table 2. COMPLEMENT FIXATION TEST OF THE STRAIN *DIs* AND THE STRAIN NAKANO (PIGEON POX) AGAINST ANTI-VACCINIA RABBIT SERUM

Antigen		Anti-vaccinia rabbit serum							Normal rabbit serum	No serum
Code	Dilution	8	16	32	64	128	256	512	8	—
DIs crude	20	4	4	4	4	4	2	0	2	0
	40	4	4	4	4	3	tr.	0	0	0
	80	4	4	4	4	tr.	0	0	0	0
	160	4	4	3	tr.	0	0	0	0	0
	320	4	1	0	0	0	0	0	0	0
	640	tr.	0	0	0	0	0	0	0	0
	1,280	0	0	0	0	0	0	0	0	0
DIs ether-treated	10	4	4	4	4	3	0	0	0	0
	20	4	4	4	1	1	0	0	0	0
	40	4	4	2	0	0	0	0	0	0
	80	4	2	tr.	0	0	0	0	0	0
	160	2	0	0	0	0	0	0	0	0
	320	0	0	0	0	0	0	0	0	0
Nakano crude	10	4	4	3	tr.	0			4	0
	20	4	4	3	tr.	0			3	0
	40	4	3	0	0	0			tr.	0
	80	2	tr.	0	0	0			tr.	0
	160	tr.	0	0	0	0			tr.	0
	320	0	0	0	0	0			0	0
Nakano ether-treated	2·5	2	tr.	0	0	0			tr.	0
	5	tr.	0	0	0	0			0	0
	10	tr.	0	0	0	0			0	0
	20	0	0	0	0	0			0	0
No antigen		0	0	0	0	0			0	0

those of the parent strain. The mutant was as labile to heat as the parent strain, showing a marked loss of infectivity after being heated at 55° C. for 30 min.

(4) *Cytopathogenicity in tissue culture:* A moderate cytopathic effect was observed in primary monkey kidney monolayers culture infected with the mutant. No cytopathic effect was observed when HeLa cells and *FL* cells were exposed to the mutant, while typical cytopathic effects accompanied by luxurious growth of virus were shown by the parent strain. A cytotoxic effect was observable with *L* cells, when infected with a large amount of either the mutant or parent strains.

(5) *Production of hæmagglutinin:* On the chorio-allantoic membrane of embryonated hen's eggs, the strain *DIs* multiplies with concomitant production of hæmagglutinin and complement-fixing antigens. However, the ratio of pock-forming units to hæmagglutinin units appeared to be slightly lower (4·6 in $\log_{10}$ units) than that observed in the case of the parent strain (5·05) (ref. 2). In HeLa cells in which no visible cytopathic effect could be obtained, a considerable amount of hæmagglutinin was nevertheless produced. This was characterized by a low ratio of pock-forming units to hæmagglutinin units (3·0 at the twenty-fourth hour of infection, as compared with 4·46 with the parent strain[3]).

These results indicate that the strain *DIs*, a minute pock-size mutant of dermovaccinia, is characterized by a reduced, or absent, virulence.

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GENETICS

Segregation-ratio Advantage of Abnormal Hæmoglobins

As a consequence of extensive investigations on abnormal hæmoglobins in Jamaica[1–4], in many instances where this was feasible family studies were undertaken. The impression was gained (as suggested in ref. 3) that some unusual inheritance patterns emerged and for this reason a statistical analysis of the available pedigrees was undertaken. Only two of the most significant deviations from the expected Mendelian ratios will be presented here.

Since only limited material was available from investigations in Jamaica, a search through the literature was undertaken. In four publications relevant family studies were reported which have been included in the analysis. It should be pointed out that any deviation from expected ratios from the Jamaican material was always present too in the same direction in these family studies. In the combined material approximately half the individuals are derived from my own investigations, the other half from ref. 5.

The results which will be discussed here come from families where one parent has the genotype *AS* and the other the genotype *AC*; these results are presented in Table 1.

The most unusual finding is the very uneven distribution of *A* and *C* genes in the offspring of families where the mother has the genotype *AC*—twenty-four *C* against seven *A* with a probability of 0·002. When the father has the genotype *AC* there is no significant difference in the number of the *A* and *C* genes in his children, with, however, a very strong preference for the daughters (as compared with the sons) to carry a *C* gene ($P = 0{\cdot}01$ for sex distribution). A further observation in accord with this is that of the nine children whom I examined from mothers with the genotype *SC*; eight inherited the *C* gene and only one the *S* gene ($P = 0{\cdot}05$).

The finding that deviations from Mendelian expectation do occur is not new. Apart from plants and *Drosophila* this is known to happen in *Sciara*, a mosquito[6]; in the mouse in relation with the *T* locus (tail formation)[7]; and has recently been described in two instances in man. Stevenson and Jennings[8] explain the preponderance of affected sons of affected fathers in the dominant condition of ectrodactyly by germinal selection. Shaw *et al.*[9] describe the marked deficiency of the autosomal dominant condition of aniridia in the offspring of aniridic parents (100 affected for 164 normal children).

The findings reported here might therefore represent the third instance of non-Mendelian segregation ratios found in man and could be explained on the basis of gametic selection or differential gametic survival.

It is suggested that the maternal or paternal gamete containing an *X* chromosome and the chromosome carrying the hæmoglobin *C* gene has a considerably better chance to occur or to result in a viable fœtus than the gamete containing the *X* chromosome and the hæmoglobin *A* gene. The gamete carrying the *Y* chromosome would not have such a selective preference.

Table 1. Distribution of Genotypes in the Offspring of Marriages where One Parent has the Genotype *AC* and the Other the Genotype *AS*

Father *AC* (10 families)							Mother *AC* (8 families)				
Genotype	Sons		Daughters	Total *A* or *C* genes with exclusion of index case			Daughters		Sons		Genotype
AA	10	*A* genes from father 13	2 1	15	*A*	7	1	*A* genes from mother 5	2 1		*AA*
AS	3		1				4			1	*AS*
AC	6	*C* genes from father 11	4	18	*C*	24	10	*C* genes from mother 20	11 3		*AC*
SC	5		13				10			8	*SC*

Another possible explanation could be post-zygotic selection, although nothing is known concerning excessive abortions in the families reported here.

If one accepts for the time being these findings as approximately correct and representative, arguments can be presented to explain the high frequencies of the *C* gene in a small area of Africa. For this purpose I will postulate that the *S* gene has a neutral value (in view of the other family investigations which are not reported here, this is probably not true) and that therefore the effect of the *C* gene would be identical in *AC–AS* and *AC–AA* matings. Starting with 0·001 per cent *AC* in a population, one can calculate, using the distribution of *A* and *C* genes presented in Table 1, that approximately thirty-two generations (about 800 yr.) will be required to bring the frequency to 10 per cent. This is a very short period of time and agrees well, therefore, with Neel's suggestion that the *C* gene must be the effect of a recent mutation, since it has not yet had time to spread beyond the Gold Coast.

One might also expect that the number of *AC* heterozygotes is not evenly distributed among the sexes; the male to female ratio should be approximately 2:3. Only little proof for such a statement is available. For work on incidence of abnormal hæmoglobins in Jamaica[1], results from pregnant women were used, possibly leading to an unusually high figure Schneider[10] finds among 505 Negro blood-donors fifteen males out of sixteen with the genotype *AC*, but her blood-donors are predominantly male. Smith and Conley[11] comment on the fact that of the fourteen cases of *AC* which they discovered, thirteen were females. Garlick and Barnicot[12] find thirteen girls against six boys with the genotype *AC* in a group of 303 Nigerian schoolchildren with otherwise equal sex distribution.

The calculations which I have used here are based on a very small body of material, and although the deviations from Mendelian ratios appear to be statistically significant, more extensive material will be required to confirm the views presented. It seems possible, however, that besides balanced polymorphism (as in the case of hæmoglobin *S* (ref. 13)) and a greater potential fertility of unions in which one parent carries a single abnormal hæmoglobin gene[14], still other forces may exist to explain the unusually high frequencies of abnormal hæmoglobins in certain populations.

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SOIL SCIENCE

Method of measuring Soil Hydraulic Conductivity *in situ*

Soil permeability or hydraulic conductivity is of great importance in the engineering design of foundations, dams, sub-surface drainage systems, irrigation systems, etc. In spite of its importance, soil hydraulic conductivity remains one of the most difficult of soil properties to assess. Laboratory methods have limitations due to the size of the samples. Considerable progress has been made in the development of methods for measuring soil hydraulic conductivity in the field when a water table is present. However, water tables are not always present in areas requiring seasonal drainage.

In order to overcome some of these difficulties, a new field method of measuring the soil hydraulic conductivity is proposed. In short, the method consists of measuring the rate of flow of water out of a cavity beneath a pipe. The method bears some resemblance to the piezometer method described by Luthin and Kirkham[1] for the measurement of soil hydraulic conductivity beneath the water table.

When water infiltrates into soil from a cavity as shown in Fig. 1, for some time after the process of infiltration has started the wetting front forms a sphere around the cavity. During this initial phase, the driving force can be considered due to the pressure gradient that exists between the cavity and a locus of points behind the wetting front. The force of gravity has negligible effect on the pattern of movement during this initial pressure phase. The duration of the pressure phase depends on the pressure in the cavity and the pressure behind the wetting front as well as the soil properties. Measurements during this initial phase should offer a method of determining the hydraulic conductivity of various soil layers or soil horizons. The following analysis is based on flow due to pressure forces alone.

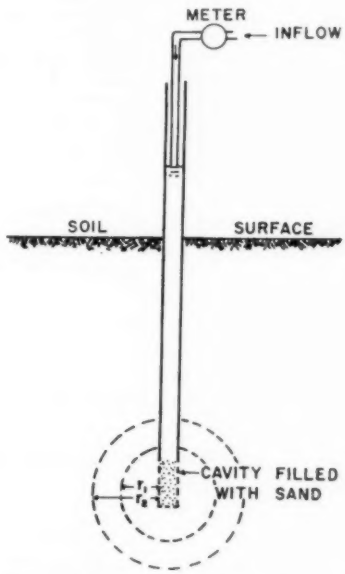

Fig. 1. Diagram of proposed method of measuring the soil hydraulic conductivity *in situ*

The assumptions which are made with regard to the flow régime are: (1), The wetting front is sharply defined and the water pressure is known at some point behind the wetting front, and (2). the soil moisture content behind the wetting front does not change appreciably with time and is close to saturation.

If Q is the volume flow per unit time out of the cavity, then as the wetting front moves radially outward:

$$Q = S4\pi r^2 dr/dt \qquad (1)$$

where S is the change in moisture content expressed as the volume of water change per unit volume of soil. The $4\pi r^2$ term represents the circumferential area of the wetting front and this is multiplied by dr to give the volume of soil. An alternative expression for the flow can be obtained by a consideration of the pressure distribution around a spherical source. The equation for the flow per unit time is given by:

$$Q = [k4\pi(p_w - p_c)]/(1/r_w - 1/r_c) \qquad (2)$$

Setting these two equations equal and solving for the hydraulic conductivity k, we have, after introducing the boundary conditions:

$$k = [1/(t_2 - t_1)][(1/3)S(r_2^3 - r_1^3)/r_w(p_w - p_c)] - 1/2[S(r_2^3 - r_1^3)/(p_w - p_c)] \qquad (3)$$

where p_c is the pressure head behind the wetting front, p_w is the average pressure head in the cavity, r_w is the equivalent radius of the cavity and $t_2 - t_1$ is the time required for the wetting front to travel from r_1 to r_2.

The above equation is used to calculate the hydraulic conductivity following the procedure outlined below. Two measurements are needed: the rate of flow from the cavity and the time for the wetting front to move from r_1 to r_2 as shown in Fig. 1. The change in moisture content, S, is calculated by $S(r_2 - r_1) = Q(t_2 - t_1)/(4\pi r^2)$, where r is taken as the average of r_1 and r_2. The progress of the wetting front from r_1 to r_2 is measured electrically.

A laboratory experiment was performed to test the validity of the assumptions used in the development of the theory. The experiment was performed in a sector tank filled with a Columbia fine sandy loam soil. Pressure measurements were made with tensiometers located at various points in the tank. The results of the tank measurement indicate that the assumptions are reasonable and the values of hydraulic conductivity obtained in the tank indicate that the method can be used to get an approximate value of hydraulic conductivity in the field.

The method is at present being developed in the field and as additional information is obtained a further report will be made.

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University of California,
Davis, California.

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Nature of Sulphate Retention by Acid Soils

Certain soils containing oxides which occur in tropical and subtropical regions are known to retain an appreciable amount of sulphate when equilibrated with dilute solutions of sulphate salts[1,4]. In our earlier work we found that the cation and anion are held in about equivalent amounts, with an increase in retention occurring as the pH was lowered or the salt concentration was increased. So far as we are aware no satisfactory explanation for all the phenomena observed has yet been given.

Recently we have found that the amount of sulphate which is held by an acid red soil increases with time of equilibration up to eight weeks (the longest period studied). Sulphate was added as K_2SO_4 and the amount retained was measured in two ways. In the first, the K_2SO_4 was labelled with sulphur-35 and its disappearance from solution was recorded. In the second, the cations in solution were measured and their sum assumed to be equal to the sulphate in solution. The first method has the disadvantage that isotopic exchange with native soil sulphate occurs and makes the results higher than the true values. The second suffers from lack of precision. Taken together, however, they show about the same trend in the retention of sulphate with time. As can be seen from Table 1, the greatest increase in sulphate held occurred during the first 24 hr.

Table 1. Effect of Time on the Retention of Sulphate from 0·01 *N* K_2SO_4 by Cecil Soil

Equilibration time	Sulphate retained (m.equiv./100 gm.) By sulphur-35	By cation sum
1 hr.	1·50	0·42
24 hr.	2·20	1·74
1 wk.	2·40	2·04
2 wk.	3·24	1·92
4 wk.	3·60	2·02
8 wk.	—	2·38

It has been found[2] that the hydrolysis of exchangeable aluminium and the formation of hydroxy-aluminium compounds proceeds at a greater rate in soil systems to which a salt has been added. In acid red soils the sulphate can exchange with some of the hydroxyl ions on the hydroxy-aluminium (or iron) polymers. The overall reaction is described as follows:

$$K_2SO_4 + Al_x(OH)_y\text{—Clay} \longrightarrow \underset{Al}{} \qquad \underset{K}{Al_x(OH)_{y-z}(SO_4)_z\text{—Clay}} + Al_2(SO_4)_3 + H^+ + OH^-$$
$$\underset{Al(OH)_3}{}$$

The replacement of hydroxyl groups from the hydroxy-aluminium groups results in the neutralization of hydrogen ions from the hydrolysis of exchangeable aluminium. Similar reactions also occur with salts of chloride and nitrate.

A reaction of this general form explains the increase in sulphate retention with increasing concentration and decreasing pH since under both these conditions hydroxyl ions would be replaced more easily. The increase in retention of sulphate with time is explained by the hydrolysis of exchangeable aluminium to a dihydroxy-aluminium species[2] and the subsequent neutralization of the hydrogen ions thus formed by replaced hydroxyl ions. This reaction provides sites on which both potassium and sulphate ions can be held.

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G. W. Thomas

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Virginia Polytechnic Institute,
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[1] Berg, W. A., and Thomas, G. W., *Soil Sci. Soc. Amer. Proc.*, **23** 348 (1959).
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FORTHCOMING EVENTS

(Meetings marked with an asterisk • are open to the public)

Monday, October 30

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. R. F. Mahler: "Glycogen Storage Diseases".•

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. J. N. Davidson: "The Biosynthesis of Polynucleotides".• (Last of two lectures.)

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. F. A. E. Pirani and Dr. G. F. Elliott: "Ballistic Missiles and Nuclear Weapons—Missile Guidance and Control".• (Third of seven lectures in War Studies. Further lectures on November 6, 13, 20, 27.)

INSTITUTION OF MECHANICAL ENGINEERS, THERMODYNAMICS AND FLUID MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion Meeting on "The Extent to Which Data can be Made Available for Engineering Design".

SOCIETY FOR VISITING SCIENTISTS (at 5 Old Burlington Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Information Theory". Chairman: Dr. A. M. Uttley. Speakers: Prof. E. C. Cherry, Prof. D. M. Mackay and Dr. H. Kalmus.

Monday, October 30—Friday, November 3

INSTITUTE OF WELDING; ROYAL INSTITUTION OF NAVAL ARCHITECTS; INSTITUTION OF ENGINEERS AND SHIPBUILDERS IN SCOTLAND; NORTH-EAST COAST INSTITUTION OF ENGINEERS AND SHIPBUILDERS; and the INSTITUTE OF MARINE ENGINEERS (at the Institute of Marine Engineers, 76 Mark Lane, London, E.C.3)—Symposium on "Welding in Shipbuilding".

Tuesday, October 31

INSTITUTE OF PETROLEUM (at the Royal Institution, 21 Albemarle Street, London, W.1), at 4 p.m.—Sir Stephen Gibson, C.B.E.: "The Birth of a Science" (Sixth Cadman Memorial Lecture).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. G. Harrison: "Functional Aspects of the Vascularization of Tissues".• (Fifth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

INSTITUTION OF MECHANICAL ENGINEERS, STEAM PLANT GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Varieties of Feed Pump Drives".

Tuesday, October 31—Wednesday, November 1

PLASTICS INSTITUTE (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2)—Conference on "Engineering with Plastics".

Tuesday, October 31—Friday, November 3

SECOND EFFLUENT AND WATER TREATMENT EXHIBITION AND CONVENTION (at the Seymour Hall, Seymour Place, London, W.1).

Wednesday, November 1

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. D. Flinn: "On Folding during 3-Dimensional Progressive Deformation"; Dr. P. E. Brown: "The Tectonic and Metamorphic History of the Pre-Cambrian Rocks of the Mbeya Region, South-west Tanganyika".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. D. Scott and Mr. D. Jamieson: "Molybdenum Disulphide as a Lubricant Additive in Rolling Contact—a Study of Compatibility with other Additives".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5.30 p.m.—Dr. F. H. Ludlam: "Giant Hail".

BRITISH INSTITUTION OF RADIO ENGINEERS (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Prof. M. R. Gavin: "The Teaching of the Theory of Transistors and other Semiconductor Devices".

INSTITUTION OF ELECTRICAL ENGINEERS; and the ROYAL AERONAUTICAL SOCIETY, LONDON JOINT GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Mr. H. J. H. Sketch: "Generation of Power in Satellites".

SOCIETY OF CHEMICAL INDUSTRY, INDUSTRIAL WATER AND EFFLUENTS GROUP (at the Royal Institution, Albemarle Street, London, W.1), at 6.45 p.m.—Dr. B. A. Southgate: "The Industrial Water and Effluents Group" (Inaugural Meeting).

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Scientific Papers.

Thursday, November 2

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion on "Mechanisms of Virus Infections" opened by Prof. Wilson Smith, F.R.S.

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. J. L. Watling: "Probability".•

CHEMICAL SOCIETY (at Queen Mary College (University of London), Mile End Road, London, E.1), at 2 p.m.—Symposium on "Electron-spin Resonance".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. J. Pritchard: "Bone Healing".• (Sixth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY COLLEGE, LONDON, and the FELDBERG FOUNDATION FOR GERMAN–ENGLISH SCIENTIFIC EXCHANGE FOR EXPERIMENTAL MEDICAL RESEARCH (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Herbert Hensel (University of Marburg): "The Electrophysiology of Thermoreceptors".• (Feldberg Foundation Lecture.)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 6 p.m.—Mr. T. Somerville: "Acoustics and the Electrical Engineer".

INSTITUTE OF REFRIGERATION (joint meeting with the REFRIGERATION SERVICEMEN'S ASSOCIATION, at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Borough Polytechnic, Borough Road, London, S.E.1), at 7 p.m.—Mr. J. E. Kumler: "Modern Designs in Thermostatic Control for Refrigeration, including Air-Conditioning, Automatic Defrosting and Heat Pump Applications".

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the BRUNEL COLLEGE OF TECHNOLOGY CHEMICAL SOCIETY, at the Brunel College of Technology, Woodlands Road, Acton, London, W.3), at 7 p.m.—Dr. J. Lewis: "Modern Developments in Co-ordination Chemistry".

Friday, November 3

MINERALOGICAL SOCIETY, CLAY MINERALS GROUP (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 11 a.m.—Autumn meeting.

INSTITUTE OF PHYSICS AND PHYSICAL SOCIETY, COLOUR GROUP (joint meeting with the LONDON SECTION OF THE SOCIETY OF DYERS AND COLOURISTS, at Burlington House, Piccadilly, London, W.1), at 6 p.m.—Mr. K. MacLaren: "A Daylight Illuminant".

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Information Theory in Relation to Biology" opened by Prof. D. M. Mackay.

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. B. Lythgoe: "The Wittig Reaction and its Application in Organic Synthesis".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. S. Brenner: "The Handing on of Genetic Information in Living Matter".

Saturday, November 4

ASSOCIATION OF CLINICAL BIOCHEMISTS, SOUTHERN REGION (at Queen Mary's Hospital, Roehampton, London, S.W.15), at 2.30 p.m.—Symposium on "Special Applications of Electrophoresis".

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Frank W. Lane: "Kingdom of the Octopus".•

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT IN BIOCHEMISTRY in St. Salvator's College—The Secretary, The University, College Gate, St. Andrews (November 4).

LECTURER, Grade I (preferably with experience in the biochemistry of proteins or nucleic acids) IN BIOCHEMISTRY—The Registrar, The University, Leicester (November 4).

LECTURER (with a good degree in zoology, and preferably research experience) IN ZOOLOGY—The Bursar, College of Advanced Technology, Gosta Green, Birmingham 4 (November 4).

LECTURERS (honours graduates in engineering or in science), to teach the subject of applied thermodynamics to students of engineering—The Secretary of University Court, The University, Glasgow (November 7).

ASSISTANT LECTURER OR LECTURER (preferably with a special interest in Africa) IN GEOGRAPHY—The Secretary, University College, Gower Street, London, W.C.1 (November 10).

ASSISTANT IN GENETICS—The Secretary of University Court, The University, Glasgow (November 10).

LECTURER (veterinary graduate with a special interest in the field of reproduction) IN THE DEPARTMENT OF ANIMAL HUSBANDRY at the Veterinary Field Station, Langford, near Bristol—The Registrar and Secretary, The University, Bristol (November 11).

SUPERINTENDENT (with administrative experience, and preferably some scientific experience) OF THE UNIVERSITY BIOCHEMICAL LABORATORY—Prof. F. G. Young, Department of Biochemistry, The University, Tennis Court Road, Cambridge (November 16).

LECTURER IN THE ELECTRONIC COMPUTATION LABORATORY of the Applied Mathematics Department—The Registrar, The University, Sheffield (November 18).

LECTURER OR ASSISTANT LECTURER (with a good honours degree or equivalent in engineering, physics or mathematics and some research experience) IN VIBRATION THEORY—The Secretary and Registrar, The University, Southampton (November 18).

BOTANIST (with a degree, preferably majoring in botany; non-graduates with a knowledge of taxonomy and herbarium experience may be considered) IN THE BOTANIC GARDEN DEPARTMENT, Adelaide, South Australia, for general herbarium, duties, determination of plants, and the supervision of botanical assistants—The Agent-General and Trade Commissioner for South Australia, South Australia House, 50 Strand, London, W.C.2 (November 20).

WYKEHAM PROFESSOR OF PHYSICS—The Registrar, University Registry, Oxford (November 23).

POSTDOCTORAL RESEARCH FELLOW IN ORGANIC CHEMISTRY at the University of Melbourne—The Registrar, University of Melbourne, Parkville, N.2, Victoria, Australia (November 25).

LECTURER (with postgraduate qualifications and research interests in experimental or social psychology) IN PSYCHOLOGY at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 27).

CHAIR OF PATHOLOGY in the Medical School, University College of Rhodesia and Nyasaland, Salisbury—The Registrar, University of Birmingham, Edgbaston, Birmingham 15 (November 30).

CHAIR OF PHYSIOLOGY in the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

LECTURER (with a degree in electrical engineering from a British university, or equivalent, preferably with first- or second-class honours, corporate member of the Institution of Electrical Engineers, and preferably with experience in teaching at university level and/or of research work) IN ELECTRICAL ENGINEERING at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, November 30).

PROFESSOR OF EXPERIMENTAL PHYSICS—The Registrar, The University, Nottingham (November 30).

REGIUS CHAIR OF SURGERY in the University of Aberdeen—The Private Secretary, St. Andrews House, Edinburgh 1 (November 30).

SENIOR LECTURER/LECTURER IN BOTANY; a SENIOR LECTURER/LECTURER (preferably with qualifications in inorganic chemistry) IN CHEMISTRY; and a SENIOR LECTURER/LECTURER IN MATHEMATICS at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

LECTURER (preferably with experience in teaching, research and in the design and construction of hydraulic works and apparatus) IN CIVIL ENGINEERING (Hydraulics) at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall London, S.W.1 (December 1).

BURNETT-FLETCHER CHAIR OF HISTORY AND ARCHAEOLOGY—The Secretary, The University, Aberdeen (December 9).

RESEARCH ASSOCIATE (with a Ph.D. in chemistry or physics) IN SPECTROSCOPY, for research in the field of chemical aspects of electron paramagnetic resonance spectroscopy—Industrial Engineering Services Research Council of Alberta, 87th Avenue and 114th Street, Edmonton, Alberta, Canada (December 15).

SENIOR LECTURER (with an honours or higher degree in science or engineering, and teaching and/or research experience in the field of physical metallurgy) IN PHYSICAL METALLURGY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (December 15).

VISITING PROFESSOR OF OCEANOGRAPHY at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1), and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (December 31).

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree in biology (including zoology), F.I.M.L.T. or equivalent qualifications, and preferably some experience in histological techniques) IN THE PHYSIOLOGY DEPARTMENT, to assist with histological investigations related to the endocrine research programme of the Department—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/22.

ASSISTANT LECTURER (Grade B) (with a degree or equivalent qualifications, teaching experience, able to teach to higher national certificate standard, and preferably some industrial experience) IN CHEMISTRY AND METALLURGY—The Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex.

ASSISTANT PROFESSOR (with two or three years postdoctoral experience in a field allied with virology, and prepared to participate in a research programme on the virology of tumours) ON CANCER RESEARCH—Dr. R. Bather, Department of Cancer Research, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

FISHERIES OFFICER (male, qualified biologist, with an honours degree in natural science, preferably zoology, and experience in research work) in Tanganyika, to investigate the ecology of inland waters in relation to fish production—Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting BCD.63/8/012.

GRADUATE (with a degree in a biological science and an interest in cytogenetics) IN THE BIOPHYSICS SECTION—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks.

LECTURER or SENIOR LECTURER IN CHEMISTRY in the Training Department—The Warden, University of London Goldsmiths' College, New Cross, London, S.E.14.

RESEARCH FELLOW IN ORGANIC CHEMISTRY—Prof. R. C. Cookson, Chemistry Department, The University, Southampton.

RESEARCH FELLOW or ASSISTANT (with a good honours degree in mathematics or engineering and preferably experience in programming for a digital computer) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS for work in the field of hypersonic gas dynamics—The Secretary and Registrar, The University, Southampton.

SECRETARY (with administrative experience in a responsible capacity in university of equivalent posts) of the College and Clerk to the Governors—The Rector, Imperial College of Science and Technology, South Kensington, London, S.W.7.

STATISTICIAN (graduate) AT THE RADIOBIOLOGICAL UNIT of the Medical Research Council, for work which includes a wide variety of physical and biological problems—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berks.

TECHNICIAN or SENIOR TECHNICIAN (with general biochemical laboratory experience and a knowledge of electronics), for duties involving assistance with research and occasional classwork and maintenance of spectrophotometers, high speed centrifuges, etc.—The Secretary, University College, Gover Street, London, W.C.1, quoting "Biochem./1".

TECHNICIAN (Scientific Assistant grade) (preferably with experience of tissue culture methods), for work on respiratory diseases of animals—The Administrative Secretary, School of Veterinary Medicine, Madingley Road, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletins of Marine Ecology (formerly *Hull Bulletins of Marine Ecology*.) Edited by Sir Alister Hardy, Dr. C. E. Lucas and R. S. Glover. No. 43, Vol. 5 : The General Hydrography of the Clyde Sea Area, Scotland. Part 1 : Description of the Area ; Drift Bottle and Surface Salinity Data. By Dr. H. Barnes and the late E. F. W. Goodley. Pp. 112–150. 11*s*. No. 44, and 45, Vol. 5 : The Multi-Depth Plankton Indicator. By R. S. Glover. A Modification of the Small Hardy Plankton Sampler for Simultaneous High Speed Plankton Hauls. By David Miller. Pp. 151–172. 6*s*. 6*d*. (Edinburgh : Scottish Marine Biological Association, 1961.) [258

University of Bristol. The Annual Report of the Agricultural and Horticultural Research Station, (The National Fruit and Cider Institute), Long Ashton, Bristol, 1960. Pp. 171 + 6 plates. (Long Ashton, Bristol : Agricultural and Horticultural Research Station, 1961.) 15*s*. [258

Agricultural Research Station : Soil Survey Research Board. Soil Survey of Great Britain, Report No. 13, 1960. Pp. iv + 57. (London : H.M. Stationery Office, 1961.) 5*s*. net. [258

Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1959–60. Tanganyika Territory—Coastal Provinces. Pp. 12. 2*s*. 6*d*. Survey of Reports. Pp. 7. 2*s*. 6*d*. (London: Empire Cotton Growing Corporation, 1961.) [258

Ministry of Agriculture, Fisheries and Food. At the Farmer's Service: a Handy Reference to Various Services available to Farmers in England and Wales. Pp. 81. (London: Ministry of Agriculture, Fisheries and Food, 1961).) [258

Other Countries

Minerva Dietologica, Vol. 1, Nr. 1 (Giugno 1961.). Pp. 72. (Rivista Trimestrale di Chimica, Biochimica, Biologia e Clinica della Nutrizione e Tecnologia della Sostanze Alimentari.) Abbonamento annuo L.3,500; Il Fascicolo L. 1,000. (Torino: *Minerva Dietologica*, Corso Bramante 83–85, 1961.) [258

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